Technical Bulletin

SOUVENIR

Brainstorming Session on

Hybrid Breeding in Onion: Innovations, Challenges and Future Prospects

16th April 2025

Venue:
NASC Complex, New Delhi

Organized by
ICAR-Directorate of Onion and Garlic Research, Pune
Horticultural Science Division, ICAR, New Delhi

In collaboration with
National Horticultural Research and Development Foundation, New Delhi

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Compiled and edited by:

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P K Gupta
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भाकृअनुप
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Prof. Sanjay Kumar Singh
FNAAS, FIAHS, FDBT
Deputy Director General
(Horticultural Science)

Foreword

It was my pleasure to participate in the Brainstorming on "Hybrid Breeding in Onion: Innovations, Challenges and Future Prospects," held on 16th April, 2025 under the aegis of the ICAR-Directorate of Onion and Garlic Research, Pune. India has just overtaken China to become the world's largest onion producer harvesting 30.21 million tons from 1.74 million hectares and had surpassed the Netherlands in exports with 1.76 million tons shipped, earning USD 499 million in 2023-24. These milestones demonstrate our nation's immense potential in the *Allium* sector. Yet, despite accounting for 22% of global onion output and utilizing 25 percent of the world's onion-dedicated cropland, our average productivity of 17.36 t/ha remains significantly lower than that of several high-yielding nations such as Korea (67.86 t/ha), Guyana (64.36 t/ha), the USA (61.28 t/ha), Australia (56.21 t/ha), Japan (47.83 t/ha) and China (22.04 t/ha). This disparity highlights the pivotal role that superior heterotic hybrids could play in elevating India's yields.

The Brainstorming Session brought together eminent researchers, policy-makers and industry stakeholders to examine these challenges and chart actionable pathways forward. I commend the organizers Dr. Amar Jeet Gupta, Dr. Vijay Mahajan, Dr. P. K. Gupta and Dr. Sudhakar Pandey for their dedication in convening this forum under the All India Network Research Project on Onion and Garlic (AINRPOG), following the recommendations of the XV Group Meeting. Their efforts, along with contributions from ICAR institutes, State Agricultural Universities and private-sector partners, ensured that discussions were both scientifically rigorous and grounded in farmers' realities.

The insights and recommendations that emerged ranging from novel molecular approaches to seed-production infrastructure and market-linkage strategies are already guiding our national hybrid breeding agenda. This souvenir is a compilation of invited

articles presented during the session, contributed by experts from ICAR institutes. I am confident that, knowledge shared in this souvenir will help accelerating the development and adoption of high-performance onion hybrids across India's diverse agro-ecologies, thereby enhancing productivity, profitability and livelihoods of the farmers.

I extend my heartfelt appreciation to all contributing authors and participants and commend the ICAR-DOGR team for their pivotal role in organizing this landmark event and compiling the outputs in this valuable volume. It is my hope that the deliberations captured herein will inspire sustained innovation and further reinforce India's leadership in global onion research and production.

(Sanjay Kumar Singh)

Date: 09.03.2026

Preface

Despite being a global leader in onion cultivation, India continues to face the challenge of low productivity, averaging only 17.36 tons per hectare, though most of the onions produced are of short-day type. This figure remains significantly below global benchmarks, where several major onion-growing regions consistently achieve yields that too high but, mostly are of long day type. The primary reasons for this yield gap include the widespread use of open-pollinated varieties (OPVs) and locally sourced seeds, which often lack genetic uniformity and quality control. In contrast, high-productivity regions across the world have successfully transitioned to 100% hybrid onion cultivation, reaping the benefits of heterosis, higher yield potential and greater uniformity in bulb traits. Hybrid onions offer clear advantages, including superior heterosis, uniformity in traits, higher yield and improved storability. Although India has advanced research in hybrid development, challenges remain in areas such as stable male sterility system development, inbred line stabilization, cost-effective seed production, biotechnological integration and wider adoption among farmers.

It was against this backdrop that the brainstorming session on Hybrid Breeding in Onion: Innovations, Challenges and Future Prospects, held on 16th April, 2025 under the aegis of ICAR, served as a timely and significant initiative. It provided a national platform for researchers, breeders, policy planners and industry stakeholders to share progress, deliberate on bottlenecks and outline actionable strategies for strengthening hybrid breeding pipelines in onion.

This souvenir captures the essence of the session through scholarly articles contributed by invited experts from leading ICAR institutes, SAUs and other national organizations. The contents of this volume span a wide thematic range covering the current status and future roadmap for hybrid breeding, male sterility systems, inbred line development, application

of biotechnological tools, hybrid vs OPV performance, cost-effective seed production strategies and molecular approaches for hybrid development.

We extend our heartfelt gratitude to all the contributors and participants whose valuable experiences and vision have shaped the content of this publication. We also acknowledge the critical role played by institutions like ICAR-DOGR, NHRDF, ICAR-IIHR, ICAR-CITH, ICAR-VPKAS, ICAR-IARI, State Agricultural Universities and seed industries in propelling onion research and development in India.

We trust this publication will serve as a useful reference and inspiration for ongoing and future efforts toward the development and dissemination of high-performing onion hybrids in the country.

Dr. Amar Jeet Gupta
Dr. Vijay Mahajan
Dr. P. K. Gupta
Dr. Sudhakar Pandey

Date: 09.03.2026

Contents

Present status, challenges and future prospects of hybrid breeding in onion

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Abstract

Onion is a high-value vegetable and staple ingredient in daily meals, not only in every Indian kitchen but also worldwide. Therefore, with the growing population, its demand continues to rise steadily. India's onion productivity remains significantly lower than the global average and other leading onion producing countries. The primary reason for low productivity is the widespread cultivation of open-pollinated varieties (OPVs), which generally yield less than hybrid varieties. Heterosis studies have shown positive significant improvements in yield, earliness, uniformity, storage quality and dry matter content. The development of commercial onion hybrids largely depends on the availability of suitable inbred lines, supported by a stable male sterile system. However, high inbreeding depression limits the availability of vigorous inbred lines and India's diverse agro-climatic conditions, present challenges in developing environmentally stable male sterile lines. Hybrid breeding became evident in India for last two decades but these challenges still persist and demands for extensive research to overcome these challenges. Development of commercial onion hybrids should prioritize the comprehensive identification of male sterile lines within the Indian onion population by using advanced biotechnological techniques. Molecular markers that differentiate cytoplasm types and are linked to the restorer of male sterility (*Ms* locus) have been identified. Expanding male sterility into elite genetic backgrounds and integrating molecular breeding including biotechnological approaches can significantly reduce the time required to develop superior onion hybrids. Furthermore, breeding efforts should focus on creating region-specific and season-specific onion hybrids to encourage widespread adoption among the farmers.

1. Introduction

Onion is a high-value vegetable farmed and domesticated for 5000 years in the ancient world (McCollum, 1976; Hanelt, 1990; Shigyo and Kik, 2008). Dating back to ancient times they are presently part of our daily meals for their nutritional value (Griffiths et al. 2002). Currently, India cultivates onions on 1.74 million hectares, producing 30.21 million tons annually. According to latest reports, India has recently overtaken China and became the largest producer of onions. Over the past 13 years production has shown a steady increase, particularly from 2016 onwards mainly due to the

expansion of cultivated area (Fig. 1). In recent years, India has surpassed the Netherlands in onion exports, shipping 1.72 million metric tons and earning 473 million USD in revenue during the 2023-24. Despite contributing 22% of the world's onion production and cultivating 25% of the global onion-growing area, India's productivity (17.36 t/ha) remains significantly lower than that of several high-yielding nations. Countries like the Republic of Korea (67.86 t/ha), Guyana (64.36 t/ha), the USA (61.28 t/ha), Australia (56.21 t/ha), China (48.29 t/ha), Chile (48.21 t/ha), Japan (47.83 t/ha), Canada (47.35 t/ha), Austria (44.68 t/ha) and Türkiye (42.98 t/ha) outperform India by a substantial margin (FAOSTAT, 2023). The higher productivity in these countries is attributed to wide spread commercialization and cultivation of onion hybrids. Globally, more than 30 onion hybrids have been developed and are widely cultivated, significantly contributing to higher yields and improved adaptability. In contrast, India's lower productivity is primarily due to its reliance on traditional open-pollinated varieties (OPVs), which are mostly grown from locally produced seeds with inherently lower yield potential compared to hybrids (Gupta et al. 2025). Furthermore, India's geographical location poses photoperiodic challenges for the development and large-scale adoption of commercial onion hybrids (Singh and Khar, 2023). The traditional varieties often exhibit genetic limitations in terms of yield potential, uniformity and stress tolerance in addition to the short shelf life and fluctuating market prices further impact the economic sustainability of onion farming (Tomar et al. 2019).

Heterosis breeding, a widely adopted strategy in many vegetables and cereal crops, offers a viable solution to overcome yield limitations in onion (Jones and Davis, 1944; Singh et al. 2023). By exploiting hybrid vigour (heterosis), hybrid onions exhibit superior traits such as higher bulb yield, uniformity, early maturity, disease resistance and improved storability (Hosfield et al. 1976; Joshi and Tandon, 1976; Hosfield et al. 1977; Dowker and Gordon, 1983, Mani et al. 1999; Gowda and Ambresh, 2014). Heterosis in onion is well-documented through numerous combining ability studies for effective hybrid breeding and heterosis exploitation (Madalageri and Bojappa, 1986; Pavlović et al. 2015; Faria et al. 2019).

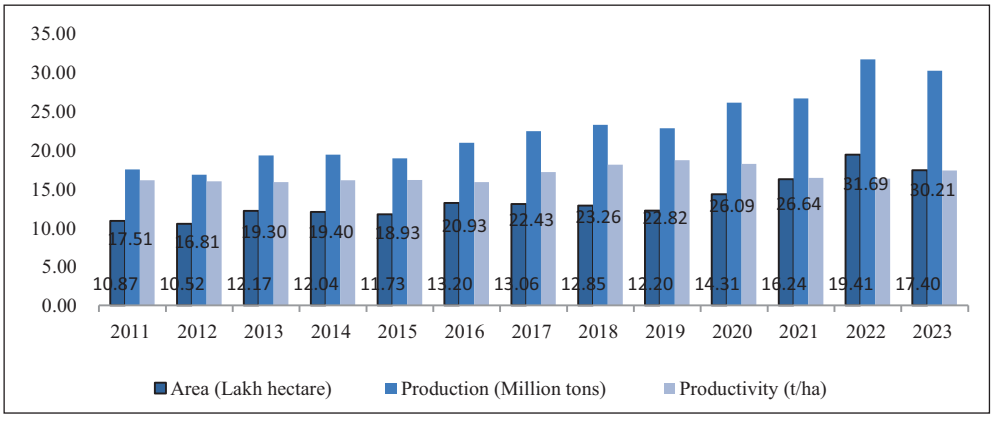


Fig. 1. Area, production and productivity of onion in India over the last 13 years (FAOSTAT, 2023)

A successful hybrid breeding in onion began after the discovery of cytoplasmic male sterility (CMS), particularly S-cytoplasm (CMS-S), which was first identified in the pedigree Italian Red 13-53 (Jones and Emsweller, 1936). This sterility is controlled by a single dominant nuclear *Ms* locus (Jones and Clarke, 1943). The first commercially grown hybrid onion, California Hybrid 1, was developed by utilizing CMS-S, where IR 13-53 (asexually propagated) was used as the female parent and crossed with an inbred line from Lord Howe Island (LHI) (Havey, 2018). This successful hybridization demonstrated the practical application of CMS in onion breeding. Later, Berninger (1965) classified another type, T-cytoplasm (CMS-T), in another sterility source Jaune Paille des Vertus (French population) where fertility restoration was governed by two complementary and one independent gene (Schweisguth, 1973). Further in 1960s and 1970s, additional reports of male sterility in various onion populations emerged from several countries, including Canada, South Africa, Holland, New Zealand, Russia, Japan, France, Hungary, The UK, Poland, Czechoslovakia, Bulgaria, and Egypt (Dowker and Gordon, 1983). These foundational studies paved the way for heterosis breeding and hybrid development in the United States and other developed countries, leading to the widespread release of onion hybrids.

Hybrid development in tropical countries where, short day onions are being cultivated is far from realization. Hybrid seed production using CMS is more stable in temperate climates, as environmental fluctuations in tropical regions can sometimes lead to partial fertility restoration, reducing hybrid seed purity (Van der Meer, 1993; Singh and Khar, 2021). Despite being a leading onion producer, India has yet to fully exploit heterosis in onions, which is a key reason for its lower productivity compared to other onion-growing nations (Gopal, 2015; Gupta and Singh, 2016; Mahajan and Gupta, 2023). Progress in hybrid onion development have been limited over the past decade, underscoring the need for more focused and prioritized research efforts. India's first recorded attempt at hybrid onion development was made in 1948 by crossing exotic male-sterile lines with local landraces. These hybrids were unsuitable for India's short photoperiod environments, as they exhibited delayed bulb initiation and poor bulb development. Since then, efforts by Indian scientists have focused on identifying and isolating male-sterile plants from local cultivars. Notable breakthroughs include identifying male-sterile plants in varieties such as Niphad-2-4-1, Nasik White Globe, IIHR-20 and Pusa Red (Patil et al. 1973; Pathak et al. 1980; Pathak, 1997).

Till now, no public sector commercial onion hybrids are available nationally in India, although the Indian Institute of Horticulture Research, Bengaluru developed two hybrid cultivars Arka Kirthiman (*MS* 65 x Sel. 13-1-1) and Arka Lalima (*MS* 48 × Sel. 14-1-1), using the male sterility mechanism in 1990. However, neither cultivar gained national popularity, underscoring the need for the development of suitable and stable inbreds for a short-day hybrid onion breeding program. Doubled haploids have emerged as the most efficient approach for developing these inbred lines, yet India remains behind in exploiting the heterosis potential in onions. Over the last two decades, Indian breeders in the public sector have focused on hybrid development, significant challenges still exist in establishing stable CMS lines, efficient restorer systems and mechanisms for large-scale hybrid seed production, leaving hybrid onion breeding in India in its nascent stages.

2. Foundation of onion heterosis breeding in India

Systematic onion research in India began in 1960. Early research efforts were initiated at IARI, New Delhi, IIHR, Bengaluru and NHRDF, Nashik, which was established in 1977 (Lawande et al. 2016). To strengthen onion and garlic research, Indian Government established the National Research Centre for Onion and Garlic (NRCOG) at Nashik, Maharashtra in 1994 and later shifted to Rajgurunagar, Pune in 1998, which was later upgraded to the Directorate of Onion and Garlic Research (DOGR) in 2009 (DOGR, 1999 & 2009). In the late 1990s, research on developing F_1 hybrids for short-day tropical onions became a priority. However, breeding for stable hybrids with high productivity and adaptability to Indian conditions has remained a challenge (Lawande et al. 2016; Gupta et al. 2015). The earliest attempts for heterosis breeding in onion date back to the late 1940s, when Sen and Srivastava (1957) used exotic male-sterile lines as female parents and locally adapted landraces as male parents. Despite multiple efforts to identify and stabilize cytoplasmic male sterility (CMS) lines from indigenous germplasm, success was limited. Some local Indian cultivars were reported to possess male sterility such as N-2-4-1 (Patil et al. 1973), Nashik White Globe (Pathak et al. 1980), Bombay White Globe (Pathak et al. 1986) and Pusa Red, IIHR-20 (DARE, 1986). In 1998-99, 65 exotic hybrids were introduced from NRI, UK, Hazra Seeds Pvt. Ltd., Israel, USA, D Palmer Seeds Nunhems, Pro Agro Seeds. However, due to their intermediate and long day photoperiodic requirement these hybrids failed to produce seeds although bulbs were formed. Meanwhile, Mani et al. (1999) successfully utilized heterosis to develop and release the landmark open-pollinated (OP) variety 'VL Piaz-3'. IIHR, Bengaluru released two F_1 onion hybrids, namely 'Arka Kirthiman' and 'Arka Lalima' in 1990. These hybrids were unable to get commercial popularity and adoption among the onion growers at national level (Khar and Saini, 2016). Gupta et al. (2016) identified two naturally occurring male sterile lines from red onions RGP-4-Sel and Arka Kalyan. Further characterization by Khar et al. 2022, Arka Kalyan reported to have 5 sterile plants of S-cytoplasm type out of 8 plants. It is necessary to characterize and determine how these lines relate to the S or T cytoplasm because they have not been described.

3. Heterosis and combining ability for hybrid breeding

Evidence of heterosis in short day onion has been well-documented, with many researchers highlighting improvements in yield, earliness, uniformity, storage quality and dry matter content. Heterosis for bulb yield and pungency (Madalageri and Bojappa, 1986), storage and early maturity (Gulumbe et al. 2018), enhanced growth (Mahala et al. 2024) have been reported. The identification of earliness enhanced by heterosis is documented by VeereGowda (1988) as well as Netrapal and Singh (1999). Positive and significant heterosis for polar and equatorial bulb diameter was recorded by Vinutha (2000) and Divakara (2001).

Bulb weight is an important character directly related to bulb yield. Joshi and Tandon (1976) noted heterosis to an extent of 72% over the mid-parent value and up to 37% over the better parent in onion hybrids. Hosfield et al. (1977) observed significant heterosis for bulb weight based on the mid-

parent. However, heterosis over better parent was significant but comparatively low, Vadivel et al. (1982) evaluated 30 hybrids and their parents. The results showed both positive and negative heterosis for bulb weight; one cross recorded significant heterosis for yield and most yield components. Aghora (1985) observed that seven crosses of onion were found significant heterosis over better parent and 13 crosses over mid-parent. VeereGowda (1988) also observed significant positive heterosis for onion bulb weight in seven crosses out of 55 crosses over better parent. Doruchowsk (1986) crossed eight male-sterile lines with eight pollen parents and heterosis was observed only for bulb weight. In a study involving 10 x 10 half diallel, out of 45 crosses, 8 had significant positive heterosis over mid-parent for bulb weight, 30 over better parent and 15 were found significant over standard check (Divakara, 2001). In hybrids of 3 male-sterile lines and 20 inbreds, various economic traits were studied by Pathak et al. (1986). Positive heterosis was observed in 9 hybrids over better parent for total bulb yield, ranged from 47.9 to 89.5%, while heterosis over best parent for marketable bulb yield was above 35% in three hybrids-*MS*1 × NER1, *MS*1 × IIHR21-l and *MS*8 × IIHR 52-1.

Line × tester cross of 3 lines (male-sterile parents) and 23 testers (fertile-male parents), thus making a total of 26 inbred parents, and their 69 F_1's along with 6 controls; quite a good number of F_1s, showed desirable heterosis over top parent for all characters except a few for maturity and neck thickness (Netrapal and Singh, 1999). All the characters revealed superiority of F_1s over standard controls. Mostly the better performing F_1s also expressed higher heterosis over better parent, top parent and standard control. Mani et al. (1999) crossed 5 red-skinned open-pollinated populations as males to 4 exotic yellow-skinned cytoplasmic male-sterile inbred lines. The resulting 20 F_1 hybrids and their 9 parents were evaluated at two locations in the Uttar Pradesh hills. The results indicated positive and significant heterosis over better parent for bulb yield in a cross inbred I_3 × L 43. In this, F_1 between inbred I_3 × L 43 was used as a base material, advanced to F_2 and was subjected to 3 cycles of mass selection for bulb yield, skin colour, shape and size. An improved, high-yielding onion strain was thus developed and designated as VL Piaz 3.

Crosses PBR 139 × AN 184 and PBR 140 × AN 187 recorded high heterosis of 45.31% and 32.83% over the better parent and 27.40% & 31.01% over the standard check (Arka Kalyan), respectively, for marketable bulb yield, and were identified as the best hybrid combinations (Shashikanth et al. 2007). According to Abubakar and Ado (2008), crosses of Red Creole × Kaharda and Kaharda × Red Creole recorded highly significant ($P<0.01$) positive high parent heterosis for disease incidence and also for fresh bulb yield. Cross, Kaharda × Ori recorded highly significant ($P<0.01$) positive high parent heterosis for bulb weight. At ICAR-DOGR, significantly higher percentage of heterosis over the checks using CMS lines was observed by Gupta and Singh (2016) and Gupta et al. (2018). F_1 hybrids developed at ICAR-DOGR exhibited more than 50% heterosis during *rabi* and more than 30% during *kharif* and *late kharif* season (Gupta and Singh, 2016). Gupta et al. (2011) evaluated different F_1 hybrids developed through male-sterile lines, and six F_1 hybrids-DOGR Hy 7, DOGR Hy 29, DOGR Hy 1, DOGR Hy 41, DOGR Hy 27 and DOGR Hy 17 were found superior over the standard check.

Since onion is a cross-pollinated crop, it offers the best opportunity for hybrid development due to heterosis. Unlike self-pollinated crops that require labour-intensive pollination, onions benefit from

natural cross-pollination facilitated by wind or insects like bees. This allows breeders to efficiently develop hybrids without manual intervention, making hybrid seed production more practical and cost-effective (Singh et al. 2023). The development of widely adopted onion commercial hybrids needs the availability of stable cytoplasmic male sterility (CMS) lines from the commercially adopted local population of onion. Studies conducted at the IIHR, Bengaluru, indicated strong cytoplasmic factor for male sterility in Indian short-day onions (Pathak et al. 1986). The cytoplasmic factors operating in Indian short-day onions were further confirmed by male-sterile lines developed at the IIHR (Rao et al. 2005). Male sterility could be successfully transferred to several breeding lines of different genetic backgrounds by backcross method, and two F_1 hybrids (hybrid 1 and hybrid 5) were identified with significant increase in bulb yield of 40.89% and 25.82% over check variety (Pathak and Gowda, 1994). The adaptation of hybrids by farmers was slow due to inherent problems associated with traditional onion-production system (VeereGowda et al. 2002). The weak parental inbred line may give a low seed yield. Therefore, combination of parental lines should be evaluated to identify superior cross combinations. Several such crosses have been made at ICAR-DOGR using 5 male sterile lines (*MS* 48A, *MS* 65A, *MS* 111A, *MS* 222A and *MS* 1600A). Sixty F_1 hybrids of red onion were developed using five male sterile lines in cross with selected twelve elite lines as pollinators *viz*., Bhima Super, Bhima Red, Bhima Raj, RGO-53, DOGR-592, DOGR-595, DOGR-1133, DOGR-1168, DOGR-1203, Bhima Kiran, Bhima Shakti and N-2-4-1 (Gupta et al. 2016b). These F_1 hybrids along with their parents and checks were evaluated for three years at ICAR-DOGR. Eight F_1 hybrids viz., DOGR Hy-1, DOGR Hy-2, DOGR Hy-3, DOGR Hy-4, DOGR Hy-5, DOGR Hy-7, DOGR Hy-8 and DOGR Hy-50 were found promising and are presently being tested at various locations through All India Network Research Project on Onion and Garlic (AINRPOG) across *kharif*, *late kharif*, and *rabi* seasons (Table 1). These hybrids are listed in Table 2 along with their characteristics. Further they were also confirmed for their purity using SSR markers (Gupta et al. 2020).

Table 1. Performance of DOGR F_1 hybrids entered in AINRPOG trials

Entry	MY (t/ha)	SH (%)	ABW (g)	Bolter (%)	DTH	Season	Storability	Bulb Shape	Bulb Colour
DOGR Hy-1	41.30	42.84	68.20	0.0	103	Rb	Good	Flat Globe	LR
DOGR Hy-2	34.96	20.91	64.50	0.0	100	Rb	Good	Globe	DR
DOGR Hy-3	34.20	16.22	68.95	0.0	95	Kh	Medium	Globe	MR
DOGR Hy-4	34.08	15.81	80.21	0.0	94	Kh	Medium	Flat Globe	DR
DOGR Hy-5	38.73	20.11	75.21	0.0	101	Kh	Medium	Globe	DR
DOGR Hy-6	41.86	17.87	70.00	0.0	108	Rb	Good	Flat Globe	MR
DOGR Hy-7	40.75	34.88	68.72	0.0	105	Kh & Rb	Very good	Flat Globe	DR
DOGR Hy-8	54.45	23.57	83.33	3.89	118	LK	Good	Flat Globe	MR
DOGR Hy-50	37.45	24.11	63.44	0.0	106	Rb	Very good	Globe	DR

Entry	MY (t/ha)	SH (%)	ABW (g)	Bolter (%)	DTH	Season	Storability	Bulb Shape	Bulb Colour
DOGR Hy-156	43.12	18.95	87.00	0.0	108	Rb	Good	Globe	LR
DOGR Hy-172	47.67	17.90	72.00	0.0	108	Rb	Good	Globe	MR
DOGR Hy-73	33.67	11.20	61.30	0.0	91	Kh	Good	Globe	DR
DOGR Hy-173	40.55	30.59	91.00	0.0	111	Rb	Good	Globe	MR
DOGR Hy-179	36.17	16.48	60.80	0.0	108	Rb	Good	Globe	MR

MY- Marketable yield; SH- Standard Heterosis, ABW- Average bulb weight; DTH- Days to harvest; Kh- *Kharif*; Rb- *Rabi*; Lk- *Late kharif*

Table 2. List of ICAR-DOGR hybrids introduced in AINRPOG trials

S. No.	Entry	Season	Reference
1.	DOGR Hy-1	*Rabi*	ICAR-DOGR Annual Report, 2012-13
2.	DOGR Hy-2	*Rabi*	
3.	DOGR Hy-7	*Rabi*	ICAR-DOGR Annual Report, 2013-14
4.	DOGR Hy-50	*Rabi*	
5.	DOGR Hy-3	*Kharif*	ICAR-DOGR Annual Report, 2014-15
6.	DOGR Hy-4	*Kharif*	
7.	DOGR Hy-5	*Kharif*	ICAR-DOGR Annual Report, 2015-16
8.	DOGR Hy-8	*Late Kharif*	ICAR-DOGR Annual Report, 2016-17
9.	DOGR Hy-6	*Rabi*	ICAR-DOGR Annual Report, 2017-18
10.	DOGR Hy-73	*Kharif*	ICAR-DOGR Annual Report, 2019
11.	DOGR Hy-173	*Rabi*	
12.	DOGR Hy-179	*Rabi*	
13.	DOGR Hy-156	*Rabi*	ICAR-DOGR Annual Report, 2021
14.	DOGR Hy-172	*Rabi*	
15.	DOGR Hy-56	*Rabi*	ICAR-DOGR Annual Report, 2022
16.	DOGR Hy-155	*Rabi*	
17.	DOGR Hy-207	*Kharif & Rabi*	ICAR-DOGR Annual Report, 2023
18.	DOGR Hy-211	*Rabi*	
19.	DOGR Hy-212	*Kharif*	
20.	DOGR Hy-169	*Kharif*	ICAR-DOGR Annual Report, 2024
21.	DOGR Hy-202	*Rabi and Kharif*	

4. Pollen behaviour of male sterile lines

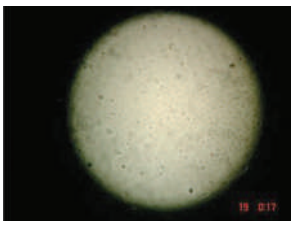
Studies indicate that cytoplasmic male sterility (CMS) in onions is linked to specific mitochondrial DNA rearrangements that disrupt normal pollen development (Patil et al. 2015; Yuan et al. 2018). The flowering behaviour of male fertile and sterile lines is presented in Fig. 2 (Gupta et al. 2016a). Pathak (1997) at IIHR, Bangalore, noted that light green anthers typically harbor male sterile pollen, while dark green anthers usually produce fertile pollen. However, these findings contrast with those of Khar and Saini (2016) and Saini et al. (2015), who observed a range of anther colours in male sterile flowers from yellowish green to dark green and found no consistent correlation between anther colour and sterility, although they did report irregular fertile pollen production, potentially due to higher temperature effects. Similarly, Manjunathagowda and Anjanappa (2020) identified male sterile lines in an Indian onion population, describing flowers with long styles, narrow stigmatic knobs, partially open perianths, and anthers that were light green to slightly yellowish translucent, shrivelled, or even empty. While earlier studies (Pathak, 1997; Santos et al. 2010; Saini et al. 2015) associated light green anthers with sterility, Santos et al. (2010) specifically linked them to male sterility, and Singh et al. (2024) later confirmed that even dark green or other coloured anthers can contain sterile pollen. Moreover, environmental factors such as temperature and plant age can cause continuous variation in anther and pollen colour, with fertile pollen exhibiting some adhesive traits and sterile pollen appearing dry and dull. In some cases, fertile flowers shown plumpy powdery anthers whereas sterile anthers showed no powdery appearance and eventually dries up without dehiscence (Fig. 2). Consequently, anther and pollen colour alone cannot reliably indicate pollen sterility. Therefore, microscopic evaluation of sterile and fertile flower is necessary for confirmation.

RGP-4-Sel male fertile umbel

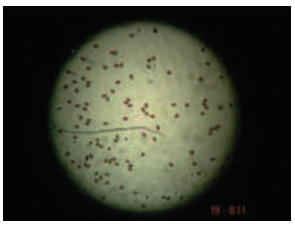
RGP-4-Sel male sterile umbel

Male fertile floret (Bhima Kiran)

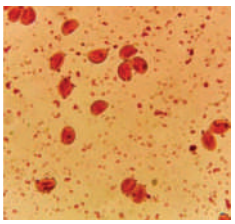
Male sterile floret (*MS* 48A)



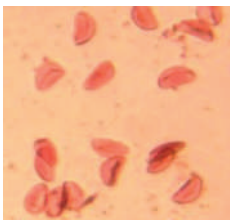
Male sterile pollens RGP-4-Sel



Male fertile pollens RGP-4-Sel



Male fertile pollens (Bhima Kiran)



Male sterile pollens (*MS* 48A)

Fig. 2. Comparison of flowering behavior of male fertile and sterile lines (Source: Gupta et al. 2016a)

5. Cytoplasm type distribution in Indian onion population

Dr. Pathak headed hybrid onion research at IIHR, Bengaluru, where his team screened 33 onion cultivars for S-cytoplasm. Only two cultivars, Red Coral (exotic) and Arka Pragati (Indian), were found to possess this cytoplasm type (Pathak et al. 1980). This discovery marked the first report of S-cytoplasm in Indian short-day onions. However, attempts to incorporate exotic male-sterile lines into breeding programs failed due to short photoperiod conditions in India which were limiting their usability (Pathak and Gowda, 1994). In India, successfully developed male-sterile hybrids exhibited sterility as reported by Pathak (1997). This led him to hypothesize that either the fertile lines carried recessive *ms* loci or that the sterile cytoplasm functioned independently of nuclear gene control. Furthermore, he proposed that Indian onion cytoplasm might be distinct from the established S and T types, representing a novel male-sterile cytoplasm. Supporting this, Havey (2000) demonstrated that the cytoplasm type in Nashik White Globe closely resembles the S type, while earlier; Havey (1993) indicated that Indian onion accessions are predominantly N-cytoplasmic, except for PI288272. Later studies provided additional insights, with Chaurasia et al. (2010) identified CMS-S cytoplasm in the Arka Kirthiman and Arka Pitambar cultivars, and Sheemar and Dhatt (2015) reporting CMS-S cytoplasm in three Indian short-day populations, while three other populations possessed only N-cytoplasm. The studies by Singh et al. (2024), Khar and Saini 2016, Khar et al. (2022) confirmed that N cytoplasm remains predominant in Indian open-pollinated varieties (OPVs), but there is a notable presence of S cytoplasm, particularly in certain commercially grown cultivars like Pusa Red and Arka Bheem. The genotyping of the *Ms* locus by Singh et al. (2024) showed that homozygous recessive (*msms)* alleles were most frequent, indicating a low availability of natural fertility restorers in the Indian population.

6. Use of molecular markers for identification of male sterile cytoplasms and restorer loci

The identification of cytoplasmic male sterility (CMS) and its corresponding fertility restoration (*Ms* locus) has been pivotal in hybrid onion breeding, with molecular markers playing a crucial role in distinguishing cytotypes and restorer alleles. Over the decades, advancements in marker technologies have significantly improved the accuracy and efficiency of hybrid development, evolving from early RFLP analyses to high-resolution SNP-based markers. S, T and N cytoplasms were distinguished by using restriction enzyme digestion of cpDNA and mtDNA (de Courcel et al. 1989; Holford et al. 1991; Havey 1993; Satoh et al. 1993). The mitochondrial gene probes *cob*, *cox1*, *cox2*, *atp6*, *atp9*, *cox3* were used to identify cytoplasm-specific polymorphisms through restriction enzyme digestion (RFLP analysis). This allowed mapping variable regions in mitochondrial and chloroplast DNA that distinguish different cytoplasm types (N, S, T) (Havey, 1995). Building on this, Havey (1993) discovered a 100-bp autapomorphic insertion in the cpDNA of N-cytoplasm, which was absent in S-cytoplasm. To develop a PCR-based marker, primers were designed to flank this insertion ensuring that amplification would produce a size-differentiated product depending on the cytoplasm type. This eliminated the need for RFLP analysis and provided a rapid, reliable method to distinguish N- and S-cytoplasms (Havey, 1995). Sato (1998) further refined molecular differentiation by focusing on *mt*DNA structural variations. Using sequencing and Northern hybridization, Sato identified a chloroplast-derived insertion upstream of the cob gene in S-cytoplasm, absent in N-cytoplasm. For

Open Pollinated (OP) populations in a mixture of N- and S-cytoplasms, molecular identification of cytoplasms can significantly reduce the number of individual pairings with a sterile tester required to identify a maintaining genotype. Determination of cytoplasmic type in onion have been shown by amplification of a 1000 bp fragment in the chloroplast genome using the polymerase chain reaction (Havey and Bark, 1994, Alcala-Sainz, 1997).

The 2000s saw the refinement of PCR-RFLP markers, with Cho et al. (2006) identifying a substitution in the *psbA* gene's Ms*pI* restriction site unique to S-cytoplasm plants. Kim et al. (2009) introduced a novel chimeric gene, *orf725*, which distinguished S and T cytoplasm types, extending the scope of molecular differentiation beyond the traditional N and S types. The 2010s marked a shift toward SNP-based markers, with Kim (2013) using CAPS markers targeting the *rps16-trnQ* region in cpDNA to distinguish between N and N_2 cytoplasm types. Von Kohn et al. (2017) further refined this by identifying 26 SNPs and an indel across 20 chloroplast genes, improving the precision of cytoplasm differentiation. More recently, Kim et al. (2019) developed SNP markers for CMS-T mtDNA, facilitating accurate differentiation among N, S, and T cytoplasms.

Simultaneously, the identification of fertility restoration genes (*Ms* locus) followed a similar trajectory of marker development. Gokce et al. (2002) pioneered this effort with RFLP marker *AOB272*, identifying single-stranded conformational polymorphisms and nucleotide variations at the *AOB272-EcoRI* site. PCR-based markers gained prominence with Bang et al. (2011), who detected a 108-bp indel polymorphism in the first intron of the OPT gene, created by tandem repeats. Further refinements by Bang et al. (2013) introduced CAPS marker *AC*ms*.1100*, which harbored an AvaII restriction site in the *Ms* fertile allele, offering precise genetic differentiation. Park et al. (2013) expanded the CAPS marker set with multiple enzyme-specific markers (*jnurf05*, *jnurf06*, *jnurf10*, *jnurf17*, *jnurf63*, and *jnurf20*), enhancing the resolution of *Ms* locus identification. The next phase of marker evolution was driven by sequencing-based approaches, as seen in Hou et al. (2015), developed a multiplex marker targeting *AcSKP1*, identifying sequence polymorphisms distinguishing fertility restorer and male-sterile lines. Kim et al. (2015) further advanced this by integrating bulked segregant analysis (BSA) with RNA sequencing (RNA-*seq*) to identify SNPs perfectly linked to the *Ms* locus, marking a shift towards high-throughput genotyping strategies.

7. Advancement of molecular markers in India

Cytoplasmic male sterility (CMS) is essential for hybrid onion production, and molecular markers have significantly reduced the time required for cytoplasm identification. Saini et al. (2015) used the *5'cob* and *orfA501* markers to differentiate N and S cytoplasm in three long-day onion populations, revealing a higher frequency of S cytoplasm across tested varieties. Similarly, Dhanya et al. (2014) employed the *orf725* marker for early-stage differentiation of male sterile and maintainer lines in Indian onion genotypes, particularly in Arka Lalima and Rose onion groups. Further advancements were reported by Khar and Saini (2016), validating cytoplasmic markers *OPT*, *MKFR*, and *accD* in short-day onion. Among 33 commercial varieties tested, two carried S cytoplasm, while *accD* emerged as a reliable marker for cytoplasm determination. Malik et al. (2017) used the cob marker in North Indian onion varieties and integrated marker-assisted selection (MAS) with phenotypic analysis to improve accuracy in identifying male sterile and maintainer lines.

More recently, Khar et al. (2022) utilized *accD* and *MKFR* markers to characterize Indian breeding lines, exotic accessions, and hybrids for their cytoplasmic makeup. Their findings confirmed the predominance of N cytoplasm in Indian onion varieties, while S cytoplasm was found mainly in exotic lines. Importantly, this study was the first to report T cytoplasm in India. Singh et al. (2024) further corroborated these findings, highlighting that *accD* and *MKFR* markers effectively identified N and S cytoplasms, while a comprehensive analysis of 35 commercial varieties reaffirmed the need for additional markers to precisely differentiate male sterile and maintainer lines (Table. 3).

Table 3. Molecular marker application for cytotype and Ms Locus identification in Indian onion genotypes

Marker/Genes	Genetic Analysis	References
Cytotype-*5'cob* & *orfA501* *Ms- OPT* & *PsaO*	Differentiated N and S cytoplasm in long-day onion populations also identified *Ms* locus; *OPT* was more effective than *PsaO*	Saini et al. (2015)
orf725	Used to distinguish sterile and maintainer genotypes at seedling stage	Dhanya et al. (2014)
Cytotype: *OSN*, *MKFR* & *accD* *Ms* locus: *OPT*, *jnurf13*, *AcSKP1* & *Ac*PMS*1*	Identified cytoplasm in short-day onions; *accD* recommended for ease of use *Ms* locus identification; most markers agreed except *OPT*	Khar and Saini (2016)
cob	Combined with phenotypic traits to isolate sterile and maintainer lines in open-pollinated varieties	Malik et al. (2017)
orf725	Validated male-sterile plants in different genotypes using phenotypic and molecular markers	Manjunathagowda and Anjanappa (2020)
jnurf20 & *PsaO*	*jnurf20* identified *Ms* locus efficiently, but *PsaO* had limitations in different backgrounds	Manjunathagowda and Selvaumar (2021)
Cytotype: *accD* & *MKFR* *Ms* locus: *Ac*PMS*1* & *AcSKP1*	Identified cytoplasm types in breeding lines, including the first report of T-cytoplasm in India *Ac*PMS*1* more reliable than *AcSKP1* for *Ms* locus identification	Khar et al. (2022)
Cytotype: *accD* & *MKFR* *Ms* locus: *Ac*PMS*1*, *AcSKP1* & *jnurf13*	Indian cultivars mostly had N cytoplasm; S cytoplasm found in some varieties; no T cytoplasm detected Most plants were homozygous recessive (*msms*) at the *Ms* locus; *OPT* marker results varied	Singh et al. (2024)

Table 4. *MS* lines identified in Indian short day type onion genotypes

***MS* lines**	**% male sterile plants reported**	**Identified/developed by**	**References**
*MS*1, *MS*8, *MS*11, *MS*39, *MS*48, *MS*65	-	Identified in Bombay White Globe, transferred to these lines by backcross breeding	Pathak et al. (1980)
VL Piaz 67	0	Molecular markers, *5'cob* and *orfA501* and acetocarmine test	Saini et al. (2015)
VL Piaz 3	2.15		
KR 1	2.15		
RGP-4-Sel	-	Acetocarmine test	Gupta et al. (2016)
Arka Kalyan			
Pusa Red	-	Three cytoplasmic (*OSN*, *MKFR* and *accD*) and four nuclear (*OPT*, *jnurf13*, *AcSKP1* and *AcPMS1*) markers	Khar and Saini (2016)
Pusa Madhavi	-		
Sel. 121-1	-		
Sel. 121-2	-		
Punjab Naroya	9.4	*Cob* cytotype specific marker, acetocarmine staining and test cross progeny scoring to identify maintainer lines	Malik et al. (2017)
Punjab Selection	7.6		
Punjab White	2.5		
COHBONC03	6.5	Black card assay and acetocarmine test and further confirmation with *orf725* marker	Manjunathagowda and Anjanappa, (2020)
COHBONC05	8.5		
COHBONC17	4.0		
COHBONC25	5.5		
DOGR-MG-2	-	*orf725* gene specific markers	Manjunathagowda et al. (2024)
VL In. 31-1A	-	-	ICAR-VPKAS
MS 111A, *MS* 222A, *MS* 100A and *MS* 1600A	-	-	ICAR-DOGR

Along with cytoplasm types, understanding the *Ms* locus, which governs fertility restoration in CMS-based hybrid breeding which is critical for developing stable and high-yielding hybrids. In this regard, several Indian studies have validated molecular markers for *Ms* locus identification. Saini et al. (2015) evaluated the performance of *OPT* and *PsaO* markers, finding that *OPT* provided better discrimination of *Ms* alleles, with 87.4% of plants showing dominant homozygous alleles (*MsMs*). Dhanya et al. (2014) also tested *orf725* for fertility restoration in Indian genotypes. Khar and Saini (2016) tested four nuclear markers *OPT*, *jnurf13*, *AcSKP1*, and *AcP*MS*1* along with cytoplasmic markers in short-day onions. They found that nuclear markers were not in linkage disequilibrium with the *Ms* locus and called for further evaluation of markers to efficiently isolate maintainer lines. Manjunathagowda and Selvaumar (2021) genotyped 72 breeding lines, concluding that while *jnurf20* was a dominant marker for the *Ms* locus, *PsaO* had limited applicability across genetic backgrounds. Manjunathagowda and Anjanappa (2020) integrated phenotypic evaluations with *orf725* to confirm male sterility. Recent efforts by Khar et al. (2022) tested *AcP*MS*1* and *AcSKP1* for *Ms* locus identification, finding that *AcP*MS*1* was more reliable than *AcSKP1*. Singh et al. (2024) provided a comprehensive molecular characterization of Indian onion varieties using *AcP*MS*1*, *AcSKP1*, and *jnurf13* markers, revealing that most commercial varieties exhibited a homozygous recessive (*msms*) genotype. However, discrepancies were noted with the *OPT* marker, necessitating further investigations to enhance precision in *Ms* locus identification. Some of the *MS* plants identified in Indian short day type onion genotypes utilizing molecular markers are listed in Table 4.

The adoption of molecular markers in Indian onion hybrid breeding has significantly advanced the identification of cytoplasmic types and fertility restoration genes, thereby facilitating the development of hybrid onion. The predominant presence of N cytoplasm and homozygous recessive (*msms*) alleles in Indian onion varieties suggests the need for continued research on S and T cytoplasm-based hybrids. While markers such as *accD*, *MKFR*, and *orf725* have proven effective for cytoplasm differentiation, the *Ms* locus requires further marker refinement to ensure precise selection of male sterile and maintainer lines. The integration of marker-assisted selection (MAS) with phenotypic evaluations will continue to play a vital role in enhancing the efficiency of hybrid breeding programs in India.

8. Doubled haploids for inbred development

Conventional heterosis breeding program demands availability of highly homozygous inbred lines to make pure heterotic combinations. Development of highly homozygous lines is very difficult in strictly cross-pollinated crop like onion. Furthermore, onion has very large genome size with highly heterozygous nature. A larger genome size means a greater number of loci and alleles, which increases the potential for genetic variation. This makes the process of achieving homozygosity more complex and time-consuming because more generations of selfing or backcrossing are required to bring alleles homozygous at all loci. A large genome with numerous deleterious recessive alleles can exacerbate the effects of inbreeding depression, leading to poor plant vigor, fertility, and bulb quality in early generations of inbreeding (Ricroch et al. 2005). A larger genome also complicates the development and application of molecular markers for marker assisted selection during inbred development. The sheer number of loci to track makes genotyping more

resource-intensive, although advances in sequencing technologies are helping to mitigate this issue. To overcome these challenges, breeders often use alternative methods such double haploids and cytoplasmic genetic male sterility. Efforts have been directed towards developing inbred lines through haploid production technology. Doubled haploids, being homozygous at all loci, serve as inbreds for hybrid production, facilitating the development of uniform and stable onion hybrids. In onion, several factors play a crucial role in achieving haploids, including genotype, flower or ovule developmental stage, pre-treatment conditions, culture media and agronomic practices. Unlike other crops, anther culture has proven less effective in onions, with gynogenesis being the preferred method for haploid induction. Studies highlight that successful in-vitro gynogenesis depends on various factors such as genotype, photoperiod, geographical origin, explant type and developmental stage, as well as culture media and environmental conditions (Anandhan et al. 2014; Khar et al. 2018; Khar et al. 2019).

For *in-vitro* culture of flower buds, the ideal developmental stage which means the buds measure approximately 3-5 mm, which corresponds to 3 to 5 days prior to anthesis (Alan et al. 2004). Onion breeders worldwide commonly utilize a straightforward, single-step protocol involving the culture of entire flower buds on induction medium until the embryos form. Research on haploid induction has predominantly focused on long-day onions, with limited studies available on short-day onions, particularly in India (Anandhan et al. 2014; Mathapati et al. 2019). Khar et al. (2018) assessed various media combinations for haploid induction using indigenous and exotic onion genotypes. Their findings highlighted that indigenous OPVs and hybrids exhibited greater responsiveness compared to exotic varieties and landraces. Among the tested media, Gamborg's B_5 medium supplemented with 2 mg/l 2,4-D and 2 mg/l BA proved most effective for haploid induction in short-day Indian onions. Mathapati et al. (2019) further reported that hormones such as Kinetin, meta-topolin, and thidiazuron had no significant impact on embryo induction.

In onions, chromosome doubling can occur either spontaneously or through induction using anti-mitotic agents. These methods enable the recovery of doubled haploids (DHs) and higher polyploids. Spontaneous chromosome doubling frequencies typically range from 10% to 30%, depending on the genotype, while induced doubling frequencies vary between 13.1% and 100%. Despite the higher efficiency of induced chromosome doubling, it has certain drawbacks, including low survival rates of explants, cytotoxic effects, vitrification, and polyploidization. Additionally, the high doses and prolonged exposure to anti-mitotic agents often result in a greater frequency of regenerants with higher ploidy levels (Jakše et al. 2003). The type and concentration of the anti-mitotic agent significantly influence the recovery of mixoploid and tetraploid plants (Alan et al. 2007). Furthermore, somatic regeneration from flower buds and second-cycle gynogenesis offer alternative approaches to produce diploid plants from mixoploid or tetraploid lines.

At IARI New Delhi, Bhima Safed and Bhima Dark Red demonstrated notable gynogenesis efficiency, with Bhima Safed recording the highest at 9.52%, followed closely by Bhima Dark Red at 9.14%. In Bhima Dark Red, a cold pretreatment for 2 days resulted in a gynogenesis efficiency of 7.33%, surpassing the control group, which achieved 6.67%. This highlights the positive influence of cold pretreatment on the gynogenesis process in this variety. For diploidization, the treatment with 500

µM colchicine for 4 hours proved to be the most effective, achieving a 75% survival rate. These findings emphasize the potential of Bhima Safed and Bhima Dark Red in gynogenesis studies and demonstrate effective protocols for enhancing gynogenesis and diploidization, contributing significantly to onion breeding programs (IARI, 2023). Very low frequency of haploid induction was observed in Indian onion genotypes (Table 5). Work on doubled haploids was initiated at ICAR-DOGR in 2005 (DOGR, 2005). Haploid induction through gynogenesis was successfully achieved in approximately 15 Indian onion genotypes. Overall gynogenic efficiency remained low, generally below 5%. Chromosome doubling in gynogenic plants was achieved using colchicine in tissue culture (DOGR, 2020). Along with this alternative approach, a haploid inducer line was developed in onion through RNAi suppression of the *AcCENH3* gene. The inducer line exhibited haploid induction in less than 5% of progeny and displayed high segregation distortion. At ICAR-DOGR, efforts are underway to induce male sterility in onion by targeting the *Ac*MS*H1* gene using CRISPR/Cas-based knockout technology (DOGR, 2022).

These preliminary studies offer a foundation for accelerating inbred line development, which is crucial for heterosis breeding in Indian onions. However, haploid induction through anther or ovule culture is highly inefficient, with low embryo or callus formation rates. Onions' biennial life cycle further slows down the evaluation of DH lines. Chromosome doubling using agents like colchicine often results in incomplete doubling, causing instability or aneuploidy. The technique is genotype-dependent, with many onion varieties showing poor responsiveness. Additionally, in vitro culture presents issues like contamination, tissue browning, and low regeneration rates. The process is also costly, requiring technical expertise and precise environmental control, which limits its widespread application in onion breeding programs.

Table 5. Haploid induction in Indian onion genotypes

Genotype	Genetic structure	Haploid frequency (%)	References
Arka Kalyan, Agrifound Dark Red, Agrifound Rose, B-780, Bhima Kiran, Bhima Red, Bhima Shakti, Bhima Shubhra, Bhima Shweta, Bhima Super, N-2-4-1, Pusa White Round, R-LKM-1, W-514	OPV	0.9-4.5	Anandhan et al. (2014)
Pusa Riddhi	OPV	1.9-5.0	Mathapati et al. (2019)
Pusa Red, Pusa Madhavi, Hisar-3, Pusa White Flat	OPV	1.34-6.8	Khar et al. (2018)
Balwan Pyaz	Landrace	1.29	
Inbred BDR	Inbred	4.05 to 5.54	DOGR (2020)

9. Current Research Initiatives

ICAR-DOGR

Over the years, extensive hybridization and evaluation programs at ICAR-DOGR have led to the development of numerous promising onion hybrids. From 2000 to 2023, over 200 hybrid combinations were evaluated, resulting in the identification of more than 15 promising hybrids, which were subsequently tested for three years under AINRPOG multi-location trials during *kharif* and *rabi* seasons. Notably, seven of these hybrids consistently yielded above the national average, with DOGR Hy-8 recording the highest productivity of 54.5 t/ha. These hybrids also demonstrated zero bolting and moderate to high bulb storability, strengthening their candidacy for wider cultivation. Early maturing hybrids such as DOGR Hy-4, Hy-7 and Hy-73 are particularly suited for short-season growing environments. To further diversify the hybrid base, 110 F_1 hybrids of red onion were developed using five CMS lines (*MS* 48A, *MS* 65A, *MS* 111A, *MS* 222A, and *MS* 1600A) crossed with 22 elite pollinators lines including 546-DR, 571-LR, KH-M-1, KH-M-2, RGP-1, RGP-2, RGP-3, RGP-4, RGP-5, 1604, 1605, 1606, 1607, 1608, 1609, 1612, 1613, 1629, 1630, 1657, 1663 and 1666. These hybrids and their parental lines were evaluated across *kharif*, late *kharif* and *rabi* seasons. Six hybrids DOGR Hy-6, Hy-73, Hy-156, Hy-172, Hy-173, and Hy-179 emerged as promising and were advanced to AINRPOG trials for multi-location assessment. All exhibited good storability, early maturity and were free from bolting and doubles. Among these, DOGR Hy-156 and Hy-179 were particularly noteworthy.

ICAR-CITH

ICAR-CITH (Central Institute for Temperate Horticulture), focuses on long-day onion improvement. Development of CMS lines project is initiated in long day onion genotypes. The CMS line development involves crossing male-sterile lines with fertile varieties like Brown Spanish. Over multiple years, seeds and bulbs are generated and evaluated through successive generations (F_1, F_2M_1, F_3M_2) using a massing method. The goal is to select plants with high yield, uniform bulbs and excellent storage traits for hybrid development (CITH, 2022).

ICAR-IIHR

Male sterility was initially identified in the variety Bombay White Globe (IIHR 20). This male sterility trait was transferred to several breeding lines with different genetic backgrounds through backcrossing. As a result, six male-sterile lines were developed-*MS*1, *MS*8, *MS*11, *MS*39, *MS*48, and *MS*65-which were later used to produce various F_1 hybrid combinations. These inbred lines were maintained through seed production in large isolation cages. The male-sterile lines (A-lines) were maintained by crossing them with their respective maintainer lines (B-lines) in cages. Heterosis and combining ability studies involving the *MS* lines and inbred parental lines led to the identification of promising hybrids, including Arka Lalima and Arka Kirthiman. Arka Lalima is a CMS based F_1 Hybrid medium sized bulbs with globe shape and firm texture. Field tolerance to diseases and pests. Suitable for *kharif* and *rabi* and recorded the bulb yield: 47 t/ha.

ICAR-IARI

At IARI, twenty test cross combinations were evaluated for their male sterile and maintainer status. Eighteen onion accessions including two commercially grown open-pollinated varieties (OPVs), four breeding lines, nine doubled haploid (DH) lines *viz.*, PMDH2, PMDH3, PMDH7, Hap2hi3.1.2, Hap2hi3.1.4, Hap2hi3.1.5, Hap2hi3.1.6, Hap2hi3.1.7, and Hap2hi3.1.8, and three hybrids were used for determining the onion cytotype and the genotype at *Ms* locus. For identification of cytoplasm, two pairs specific for identification of onion cytoplasm type *viz*., *accD*, and *MKFR* were utilized. For determination of *Ms* locus, two primers *viz.*, *AcSKP1* and *Ac*PMS, found to be in complete linkage disequilibrium with the onion nuclear *Ms* locus, were employed. A mixture of normal and sterile cytoplasm was identified (IARI, 2021). A set of 40 advanced breeding lines of long day onion (red, yellow and white) was purified and maintained (IARI, 2023).

PAU, Ludhiana

At PAU, Ludhiana, the first hybrid POH-1 was developed over a decade-long research initiative led by Dr. A.S. Dhatt and his team at PAU. This hybrid is notable for its 'flowerless' characteristic, which effectively eliminates the issue of bolting-a common problem where premature flowering adversely affects bulb development. The absence of bolting in POH-1 ensures larger bulb sizes and enhances overall yield.

10. Released varieties of onions in India

S. No.	Variety	Special feature	Recommended season	Organization	Year of release
1.	N-53	Red	*kharif*	Agri. Dept., MS	1975
2.	N-2-4-1	Red	*rabi* and *LK*	Dept. of Agri. (MS)	1985
3.	N-257-9-1	White	*rabi*	Dept. of Agri. (MS)	1985
4.	Baswant-780	Red	*kharif*	MPKV, Rahuri	1989
5.	Phule Safed	White	*LK* and *rabi*	MPKV, Rahuri	1994
6.	Phule Suvarna	Yellow	*rabi* and *LK*	MPKV, Rahuri	2001
7.	Phule Samarth	Red	*LK*	MPKV, Rahuri	2006
8.	Pusa White Flat	White	*rabi*	IARI, New Delhi	1975
9.	Pusa White Round	White	*rabi*	IARI, New Delhi	1975
10.	Early Grano (LD)	Yellow	*rabi*	IARI, New Delhi	1975
11.	Brown Spanish (LD)	Brown	*Hills*	IARI, RRS, Katrain, Kullu (HP)	1975
12.	Pusa Red	Red	*rabi*	IARI, New Delhi	1975

S. No.	Variety	Special feature	Recommended season	Organization	Year of release
13.	Pusa Ratnar	Red	*rabi*	NBPGR, New Delhi	1975
14.	Pusa Madhavi (L-120)	Red	*rabi*	IARI, New Delhi	1987
15.	Selection 126	Brown	*rabi*	IARI, New Delhi	2012
16.	Arka Pragati	Red	*kharif* and *rabi*	IIHR, Bengaluru	1984
17.	Arka Niketan	Red	*rabi* and *LK*	IIHR, Bengaluru	1987
18.	Arka Kalyan	Red	*kharif*	IIHR, Bengaluru	1987
19.	Arka Pitamber	Yellow	*rabi*	IIHR, Bengaluru	2006
20.	Arka Bindu	Red	*kharif, LK* & *rabi*	IIHR, Bengaluru	2006
21.	Arka Ujjwal (multiplier onion)	Red	*rabi*	IIHR, Bengaluru	2010
22.	Arka Swadista	White	*rabi*	IIHR, Bengaluru	2010
23.	Arka Vishwas (Rose onion)	Dark red	*kharif* and *rabi*	IIHR, Bengaluru	2011
24.	Arka Sona	Yellow	*rabi*	IIHR, Bengaluru	2011
25.	Arka Bheem (tri parental synthetic)	Red	*rabi*	IIHR, Bengaluru	2011
26.	Arka Akshay (tri-parental synthetic)	Dark red	*rabi*	IIHR, Bengaluru	2011
27.	Arka Lalima (F_1)	Red	*kharif* and *rabi*	IIHR, Bengaluru	1998
28.	Arka Kirthiman (F_1)	Red	*kharif* and *rabi*	IIHR, Bengaluru	1998
29.	Arka Yojith	White	*rabi*	IIHR, Bengaluru	-
30.	Hissar-2	Red	*rabi*	HAU, Hissar	1976
31.	HOS-1	Red	*rabi*	HAU, Hissar	2006
32.	Hissar Onion-3	Bronze	*rabi*	HAU, Hissar	2010
33.	Hissar Onion-4	Rose red	*rabi*	HAU, Hissar	2016
34.	Agrifound Light Red	Red	*rabi* and *LK*	NHRDF, Nashik	1988
35.	Agrifound Dark Red	Red	*kharif*	NHRDF, Nashik	1996
36.	NHRDF Red (L-28)	Red	*rabi*	NHRDF, Nashik	2006
37.	NHRDF Red-2 (L-355)	Red	*rabi*	NHRDF, Nashik	2012
38.	NHRDF Red -3 (L-625)	Light bronze	*rabi*	NHRDF, Nashik	2004
39.	NHRDF Red-4 (L-744)	Dark red	*rabi*	NHRDF, Nashik	2012

S. No.	Variety	Special feature	Recommended season	Organization	Year of release
40.	NHRDF Fursungi (L-819)	Red	*rabi*	NHRDF, Nashik	2015
41.	Agrifound Rose	Red	*Rabi*	NHRDF, Nashik	1987
42.	Agrifound Red (Multiplier)	Red	*kharif* and *rabi*	NHRDF, Nashik	1987
43.	Agrifound White	White	*rabi*	NHRDF, Nashik	1994
44.	VL-67 (Long Day)	Red	Hills	VPKAS, Almora	1973
45.	VL-3 (Long Day)	Red	Hills	VPKAS, Almora	1990
46.	Udaipur 101	Red	*rabi*	RAU, Rajasthan	-
47.	Udaipur 102	White	*rabi*	RAU, Rajasthan	-
48.	Udaipur 103	Red	*rabi*	RAU, Rajasthan	-
49.	PKV White	White	*rabi*	PDKV, Akola	2009
50.	GWO-1	White	*rabi*	GAU, Junagadh	2000
51.	GJRO-11	Red	*rabi*	GAU, Junagadh	2016
52.	GJWO-3	White	*rabi*	GAU, Junagadh	2016
53.	Kalyanpur Red Round	Red	*rabi*	CSAUAT, Kanpur	1983
54.	Punjab Selection	Red	*rabi*	PAU, Ludhiana	1973
55.	Punjab Red Round	Red	*rabi*	PAU, Ludhiana	1993
56.	Punjab-48 (S-48)	White	*rabi*	PAU, Ludhiana	1978
57.	Punjab White	White	*rabi*	PAU, Ludhiana	1998
58.	Punjab Naroya (PBR-5)	Red	*rabi*	PAU, Ludhiana	1997
59.	CO-1 (Multiplier)	Red	*kharif* and *rabi*	TNAU, Coimbatore	1965
60.	CO-2	Red	*kharif* and *rabi*	TNAU, Coimbatore	1978
61.	CO-3	Red	*kharif* and *rabi*	TNAU, Coimbatore	1982
62.	CO-4	Red	*kharif* and *rabi*	TNAU, Coimbatore	1984
63.	CO-5	Pink	*kharif* and *rabi*	TNAU, Coimbatore	2001
64.	MDU-1	Red	*rabi*	TNAU, Coimbatore	1982
65.	CO-6	Pink	*kharif* and *rabi*	TNAU, Coimbatore	2020
66.	Rajasthan Onion-1 (RO-1)	Red	*rabi*	RARI, Durgapura	2004
67.	Arpita (RO-59)	Red	*rabi*	RARI, Durgapura	2005
68.	RO-252	Red	*rabi*	RARI, Durgapura	2011

S. No.	Variety	Special feature	Recommended season	Organization	Year of release
69.	Bhima Super	Pink	*kharif, LK* & *rabi*	ICAR-DOGR, Pune	2016
70.	Bhima Raj	Dark red	*kharif* and *rabi*	ICAR-DOGR, Pune	2008
71.	Bhima Red	Med Red	*kharif* and *LK*	ICAR-DOGR, Pune	2015
72.	Bhima Shakti	Med Red	*LK* and *rabi*	ICAR-DOGR, Pune	2019
73.	Bhima Kiran	Light red	*rabi*	ICAR-DOGR, Pune	2015
74.	Bhima Shweta	White	*kharif* and *rabi*	ICAR-DOGR, Pune	2015
75.	Bhima Shubhra	White	*kharif and LK*	ICAR-DOGR, Pune	2015
76.	Bhima Dark Red	Dark red	*kharif*	ICAR-DOGR, Pune	2015
77.	Bhima Safed	White	*kharif*	ICAR-DOGR, Pune	2018
78.	Bhima Light Red	Light red	*rabi*	ICAR-DOGR, Pune	2018
79.	Bhima Gulab (RGP-3)	Dark red	*Kharif*	ICAR-DOGR, Pune	2024
80.	Bhima Kesar (DOGR-1625)	Dark red	*Kharif*	ICAR-DOGR, Pune	2024
81.	Bhima Early (DOGR-1203-DR)	Dark red	*Rabi*	ICAR-DOGR, Pune	2024
82.	Bhima Jyoti (DOGR-1550-Agg) (Multiplier onion)	Med red	*Rabi*	ICAR-DOGR, Pune	2024
83.	Bhima Jata (DOGR-1546-Agg) (Multiplier onion)	Pink	*Rabi*	ICAR-DOGR, Pune	2024
84.	DOGR-HT-3 (Bhima Prasanskaran)	White	*Rabi*	ICAR-DOGR, Pune	2024
85.	DOGR-HT-4 (Bhima Nirjala)	White	*Rabi*	ICAR-DOGR, Pune	2024
86.	DOGR-361 (Bhima Ujjwala)	White	*Rabi*	ICAR-DOGR, Pune	2024

11. Challenges in hybrid breeding

1. Identification of male sterile and their maintainers from local and commercial Indian population

In Indian onion genotypes, fertile normal (N) cytoplasm along with homozygous recessive (*msms*) nuclear constitution is predominantly observed (Khar *et al*. 2022). Moreover, since normal cytoplasm has dominated onion population in India, the restorer *Ms* locus is very rarely observed and finding corresponding *Ms* locus for maintenance/hybrid production is very tedious. Further research is necessary to identify better markers or to fine-map the *Ms* locus for more precise breeding efforts in Indian onion varieties. Without a marker in complete linkage disequilibrium with

the *Ms* locus, breeders cannot fully rely on these markers to identify fertility-restoring lines during hybrid breeding.

1.1 Low Frequency of male-sterile lines

Studies have shown that male-sterile lines occur at low frequencies in Indian onion populations, typically ranging between 2.7% and 8.5%. This limited availability makes it difficult to identify and isolate stable male-sterile lines for breeding programs.

1.2 Cytoplasmic diversity and complexity

Indian onion populations exhibit diverse cytoplasm types, including N, S and, recently T cytoplasm. Differentiating and characterizing these cytoplasm types is challenging, as they may influence the stability of male sterility. The unique cytoplasm types in Indian onions often deviate from those found in other regions, complicating their classification and utilization.

1.3 Linkage disequilibrium of markers

The identification of the *Ms* locus, which governs male sterility, relies on molecular markers such as AcSKP1 and AcPMS. While these markers show complete linkage disequilibrium with the *Ms* locus, variations in marker expression across genotypes can reduce their effectiveness in accurately identifying male sterility and maintainers.

1.4 Environmental instability of male sterility

Male sterility in Indian onions is often unstable under tropical conditions. Variability in photoperiods and temperatures affects the expression of sterility, making it difficult to consistently reproduce male-sterile phenotypes.

1.5 Lack of uniformity in local populations

Indian onion populations, particularly landraces and local cultivars, exhibit high genetic heterogeneity. This diversity, while valuable for breeding, complicates the identification of stable maintainers and consistent male-sterile lines.

1.6 Limited molecular data

Molecular studies on Indian onion populations remain limited compared to those in other onion-growing nations. A lack of comprehensive molecular markers and genomic resources hinders precise identification and classification of male-sterile lines.

2. Production of suitable and stable inbreds

The development of inbred lines is a critical step in onion breeding, particularly for the production of hybrid varieties. Onions are predominantly cross-pollinated due to their protandrous nature, which requires specialized techniques to achieve homozygosity. Conventional crossing using inbred lines is often considered an alternative strategy when stable male sterility systems are not available or are difficult to implement. Inbred lines can be crossed to generate hybrid offspring with desirable traits,

such as improved yield, disease resistance, or better quality. While this approach may be less efficient or more time-consuming than using male sterility systems, it can still be effective in producing hybrids, especially in crops like onions, where hybrid vigour is important for commercial production. However, producing stable inbred lines in onions is challenging due to their high genetic heterozygosity, self-incompatibility and susceptibility to inbreeding depression. Their biennial life cycle further prolongs the process, as it takes two years to complete one generation. Additionally, onion flowering and seed production are highly sensitive to environmental factors, and self-pollination often results in low seed set and viability. These factors, combined with the need for controlled pollination to avoid contamination, make inbred line development in onions a time-consuming and labour-intensive process.

3. Challenges in double haploid technique for inbred development

Haploid induction through anther or ovule culture is highly inefficient, with low embryo or callus formation rates. Onions' biennial life cycle further slows down the evaluation of DH lines. Chromosome doubling using agents like colchicine often results in incomplete doubling, causing instability or aneuploidy. The technique is genotype-dependent, with many onion varieties showing poor responsiveness. Additionally, in vitro culture presents issues like contamination, tissue browning and low regeneration rates. The process is also costly, requiring technical expertise and precise environmental control, which limits its widespread application in onion breeding programs.

4. Biennial crop nature

Requires two years to complete its life cycle. In the first year, the plant focuses on vegetative growth, producing green leaves and developing an underground bulb, which stores energy. This bulb is typically harvested at the end of the first growing season for culinary use. If left in the ground or replanted, the onion enters its second year, where it transits to its reproductive phase, sending up a flowering stalk called a scape. The flowers produce seeds after pollination, completing the cycle.

5. Highly cross-pollinated nature and strict isolation distance

Onion being highly cross-pollinated crop and requires minimum isolation distance of about 1.5 km for commercial seed production to maintain purity.

6. Cross-ability with wild sources is difficult

Cross-ability between cultivated onion and wild onion species is generally difficult due to genetic, reproductive and ecological barriers.

7. Significant storage losses of bulbs

Onion storage losses are a significant concern due to the susceptibility of bulbs to rotting, sprouting and physiological degradation, which can lead to the loss of both germplasm and commercial stock. Factors such as improper storage conditions (high humidity, fluctuating temperatures and poor ventilation) can exacerbate these losses.

8. Limited seed viability under ambient conditions

The viability of onion seeds under ambient conditions is relatively short, typically lasting only 15 to 18 months. This limited seed viability is due to the high moisture content in onion seeds and their sensitivity to environmental factors such as temperature and humidity. Seeds stored under ambient conditions, especially in warm and humid climates, tend to lose their germination capacity rapidly due to the breakdown of essential seed components and increased susceptibility to fungal infections.

9. Extreme sensitivity to abiotic stresses and climate change

Onion is highly sensitive to abiotic stresses such as temperature extremes, drought, salinity, and waterlogging, making it vulnerable to the impacts of climate change. These stresses can significantly reduce bulb quality, size and yield.

12. Future prospects of onion hybrid breeding

The limited availability of popular onion hybrids in India originates from insufficient technological advancements and research in onion breeding. Additionally, well-structured policies and strategic decision-making frameworks are essential for promoting hybrid onion development in the country. Many onion researchers suggest that the currently available male sterile lines lack environmental stability, which can compromise hybrid purity and vigour. Since male sterility in onions is influenced by factors such as temperature fluctuations and photoperiod changes, thorough multi-location and multi-season trials are necessary to identify stable male sterile lines before hybridization. Furthermore, an ideal maintainer line (B-line) should closely resemble the male sterile line, differing only in its fertility trait. Any fertility restoration genes in the maintainer line can interfere with complete male sterility, leading to compromised hybrid quality. The use of PCR-based molecular markers such as *AcSKP1* and *AcP*MS*1* can effectively help in detecting and eliminating these unwanted fertility restoration genes (Khar et al. 2022).

Additionally, the incorporation of doubled haploid (DH) technology in onion breeding can accelerate the development of inbred lines (Poornima et al. 2024). Given that onions have a biennial life cycle, requiring two years to produce seeds, conventional selfing and selection methods can be time-intensive and may result in inbreeding depression. Expanding male sterility into elite genetic backgrounds and integrating molecular breeding and biotechnological approaches can significantly reduce the time required to develop superior onion hybrids. Furthermore, breeding efforts should focus on creating region-specific and season-specific onion hybrids to encourage widespread adoption among farmers. There is also a growing demand for hybrids with processing-specific traits, such as high total soluble solids (TSS) and uniform large white bulbs, particularly for onion dehydration industries (Raghu, 2024). To achieve these goals, establishing multi-institutional, time-bound collaborative projects at national and international levels is crucial for accelerating onion hybrid development in India.

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Onion Hybrids: An ICAR-DOGR Perspective

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Abstract

Onion is a cornerstone of Indian agriculture and trade, yet its productivity lags behind major onion producing nations due to reliance on open pollinated varieties and limited exploitation of hybrid vigor. Since its establishment in 1994 (upgraded to ICAR-DOGR in 2008), the Directorate of Onion and Garlic Research has spearheaded heterosis breeding, cytoplasmic male sterility (CMS) development, doubled–haploid technology and biotechnological interventions to accelerate hybrid onion development. ICAR-DOGR has released 18 open pollinated varieties. However, despite its efforts, hybrids have not been officially released yet. Beginning in the late 1990s, ICAR-DOGR evaluated exotic hybrids that were ultimately found unsuitable for Indian conditions due to photoperiod sensitivity. Later on, focus then shifted to indigenous hybrid development using male sterility systems, possessing CGMS like MS 48A, MS 65A, MS 111A, MS 222A, MS 1600A and MS 100A. The institute has conserved for 1,523 onion accessions including landraces and wild species, forming a robust genetic resource for breeding. Though ICAR-DOGR has developed over 15 promising F_1 hybrids with notable improvements in the traits of yield, storability, early maturity and resistance to bolting and diseases which were validated through multi-location trials under the AINRPOG. Biotechnological advancements, such as the use of SSR markers for CMS detection, hybrid purity and fertility restoration, have enhanced breeding efficiency. A significant achievement includes doubled haploids (DH) production through gynogenesis and CRISPR/Cas9-mediated mutagenesis of the *AcMSH1* gene, enabling faster development of homozygous lines. Ongoing efforts include CMS introgression into elite lines, evaluation of 70 new crosses under the CRP on hybrid technology in onion and training initiatives for farmer-led hybrid seed production. Emphasis is placed on genetic base broadening using wild *Allium* species and long-day onion introgressions. With these integrated strategies, ICAR-DOGR aims to increase onion highest in productivity and boost exports by 2050. The article underscores that hybrid onion development is pivotal to transforming India's onion sector with ICAR-DOGR's innovations laying a solid foundation for nation's future growth.

1. Introduction

Onion is a high-value vegetable widely cultivated in India, providing substantial income to farmers and playing a vital role in agricultural trade and exports. Onions were introduced to India around

600 BC from Central Asia and have been cultivated since ancient times, becoming an integral part of Indian agriculture and cuisine (Gupta et al. 2025). The Indian onions are famous for their pungency and are available round the year (Mahajan and Gupta, 2023). There is a lot of demand for Indian Onion in the world. In 2023-24, India exported 1.72 million tons of fresh onions worldwide, generating revenue of 473 million USD (APEDA, 2024). India has produced 30.21 million tons of onions on a 17.4 lakh hectares area surpassing China in 2023 (FAOSTAT, 2023). Over the past 13 years production has shown a steady increase, primarily due to the expansion of cultivated area rather than the adoption of high-yielding varieties (Fig. 1). Therefore, India's productivity (17.36 t/ha) remains significantly lower than that of several high-yielding nations. Countries like the Republic of Korea (67.86 t/ha), Guyana (64.36 t/ha), the USA (61.28 t/ha), Australia (56.21 t/ha), China (48.29 t/ha), Chile (48.21 t/ha), Japan (47.83 t/ha), Canada (47.35 t/ha), Austria (44.68 t/ha), and Türkiye (42.98 t/ha) outperformed India by a substantial margin (FAOSTAT, 2023). The higher productivity in these countries is attributed to wide spread commercialization and cultivation of onion hybrids. The primary reasons for low onion productivity in India are the widespread cultivation of open-pollinated varieties (OPVs), often grown from locally sourced seeds, and the limited availability of high-yielding F_1 hybrids at the national level (Singh and Khar, 2023).

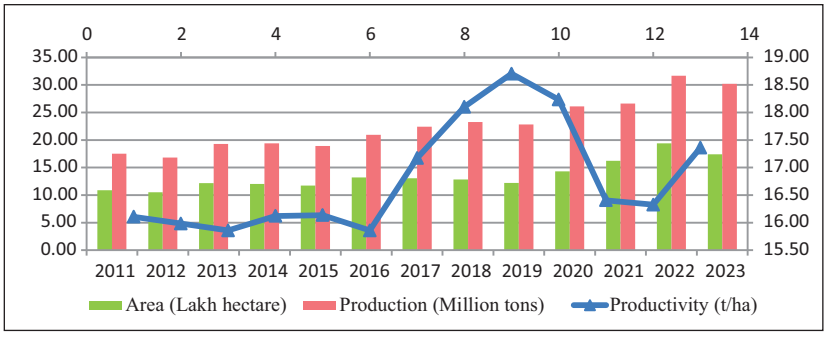


Fig. 1. Area, production and productivity of onions in India over the past 13 years ***(Source: FAOSTAT, 2023)***

In India, onion is grown in three seasons, i.e. *kharif*, *late kharif* and *rabi*. Main crop is in *rabi* which covers about 50% of production; whereas, 20% is from *kharif* and 30% from *late kharif*. Maharashtra, Karnataka, Gujarat, Bihar, Madhya Pradesh, Rajasthan, Andhra Pradesh and Tamil Nadu are main onion-growing states. In general, apart from North-Eastern states and Kerala, all other states grow onion. Country's 26% area and 29% production alone come from Maharashtra. About 90% export of onion is from Maharashtra. From May to November, stored onions are used for domestic as well as export market. November to December, *kharif* onion is available in the market, whereas from January to March *late kharif* crop from Maharashtra is available but, there is critical shortage in arrivals of onion in the market during November to January. The productivity in *late kharif* and *rabi* is around 25 tons per hectare, whereas in *kharif* season it is 8-10 tons per hectare. Cloudy weather and persistent drizzling during the *kharif* season create favourable conditions for diseases such as anthracnose and bulb rot, significantly reducing productivity. In *rabi* season high incidence of thrips aggravates the problem of purple blotch and *Stemphylium* blight, which again pulls down the productivity.

Heterosis breeding, a widely adopted strategy in many vegetable and cereal crops, offers a viable solution to overcome yield limitations in onion (Jones and Davis, 1944). By exploiting hybrid vigour

(heterosis), hybrid onions exhibit superior traits such as higher bulb yield, uniformity, early maturity, disease resistance and improved storability (Hosfield et al. 1976; Joshi and Tandon, 1976; Hosfield et al. 1977; Dowker and Gordon, 1983, Mani et al. 1999; Gowda and Ambresh, 2014). Heterosis in onion is well-documented through numerous combining ability studies for effective hybrid breeding and heterosis exploitation (Madalageri and Bojappa, 1986; Pavlović et al. 2015; Faria et al. 2019). Hybrid development in tropical countries where short day onions are being cultivated is far from realization. Hybrid seed production using CMS is more stable in temperate climates, as environmental fluctuations in tropical regions can sometimes lead to partial fertility restoration, reducing hybrid seed purity (Van der Meer, 1993; Singh and Khar, 2021). Despite being a leading onion producer, India has yet to fully exploit heterosis in onions, which is a key reason for its lower productivity compared to other onion-growing nations (Gopal, 2015; Gupta and Singh, 2016; Mahajan and Gupta, 2023). Exotic hybrids *viz*., Matahari, Linda Vista, Nun-3001, Nun-3800, Rio Tinto, Flare, Orient, BSS-227, BS-441 and BSS-442 are available in the market but exotic hybrids are poor in storage and produce smaller bulbs under tropical conditions, thus unsuitable for short photoperiodic environments of India. Progress in hybrid onion development have been limited over the past decade, underscoring the need for more focused and prioritized research efforts.

Recognizing the importance of onion, to increase its production and cultivation, Indian Government established National Research Centre for Onion and Garlic (NRCOG) in 1994 at Nashik, Maharashtra, marked a significant milestone of onion and garlic research in India. In 1998, the centre was relocated to Rajgurunagar, Pune to enhance research infrastructure and expand its activities. That same year (1998-99), hybrid breeding programs were formally initiated to develop high-yielding, disease-resistant and stress-tolerant onion hybrids. In 2008, the centre was upgraded to the Directorate of Onion and Garlic Research (ICAR-DOGR), broadening its mandate to national-level research and development. Since then, ICAR-DOGR has been continuously engaged into improvement of onion and garlic production. This article traces the evolution of hybrid onion breeding at ICAR-DOGR from its inception in 1994 to 2024, highlighting key national programs, breeding achievements, biotechnological interventions, and future prospects.

2. Establishment and Profile of ICAR-DOGR

The journey of organized research on onion and garlic in India began with the establishment of the National Research Centre for Onion and Garlic (NRCOG) at Nashik in 1994. However, due to logistical challenges such as the remoteness of the farm location and limited irrigation facilities, the centre was relocated in June 1998 to the Central Potato Research Station (CPRS) regional station at Rajgurunagar, Pune, which offered better infrastructure and research potential. The relocation marked a turning point in the centre's focus, with immediate emphasis placed on hybrid development research (DOGR, 1999).

As research and development activities expanded significantly over the years, the centre was upgraded to the Directorate of Onion and Garlic Research (ICAR-DOGR) in December 2008, further strengthening its mandate and national leadership in onion and garlic improvement programs (DOGR, 2008). ICAR-DOGR has been at the forefront of hybrid development in onion, initiating several dedicated research projects to harness heterosis and enhance productivity.

To date, the centre has released 18 onion varieties, including multiplier onion varieties that are suited for various seasons and regions (Table 1 & Fig. 2). However, despite concerted efforts toward hybrid development, no onion hybrid has yet been officially released. Continued efforts are ongoing to overcome challenges in hybrid seed production and CMS stability to realize the full potential of hybrid onions in India.

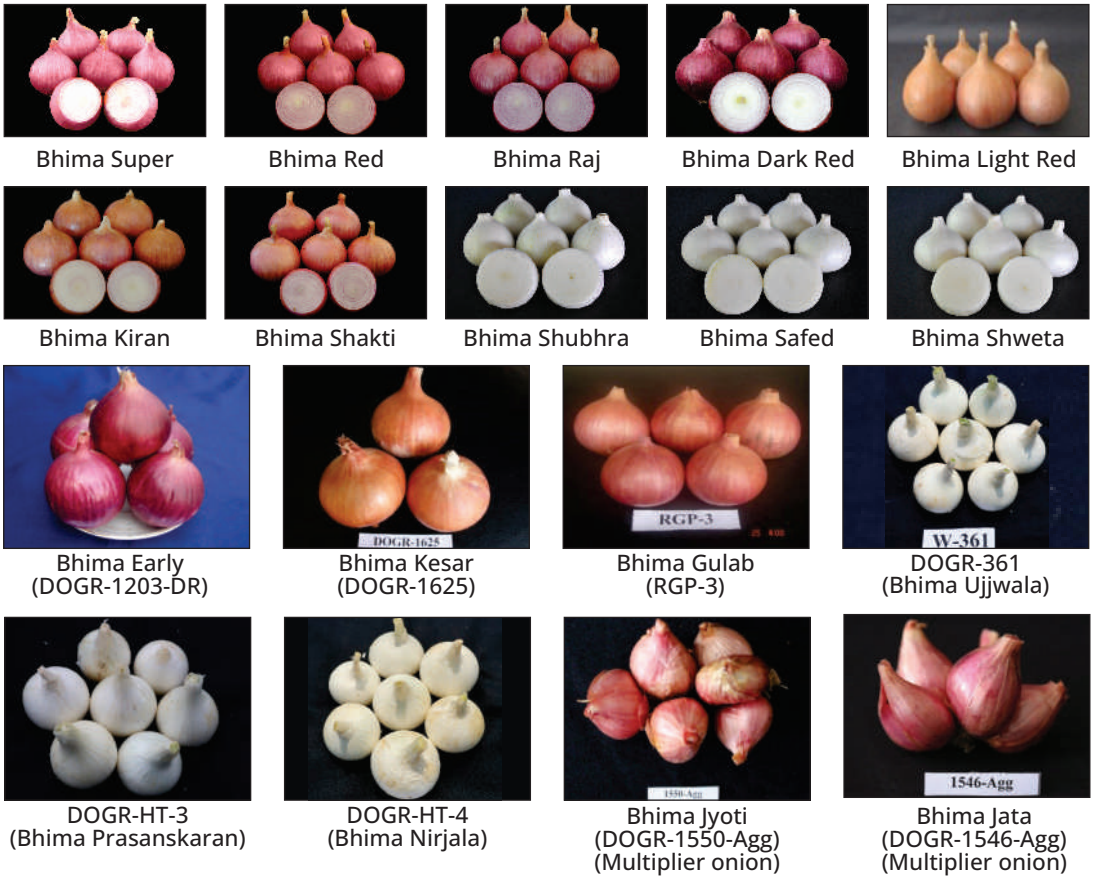


Fig. 2. Onion varieties released by ICAR-DOGR at national level

Table 1: List of released varieties of onion by ICAR-DOGR at national level

S. No.	Released varieties	Year of Release/ Notification	Season type	Bulb characteristics
1	Bhima Raj	2008	*Kharif* and *LK*	Dark red, globe shaped and thin neck bulbs
2	Bhima Shweta	2015	*Kharif* and *Rabi*	White colour, flat-globe shaped and thin neck bulbs

Table 1: List of released varieties of onion by ICAR-DOGR at national level

S. No.	Released varieties	Year of Release/ Notification	Season type	Bulb characteristics
3	Bhima Shubhra	2015	*Kharif* and *LK*	White colour, globe shaped and thin neck bulbs
4	Bhima Dark Red	2015	*Kharif*	Dark red, flat-globe shaped and medium neck bulbs
5	Bhima Red	2015	*Kharif, LK & Rabi*	Medium red, globe shaped, thin neck bulbs
6	Bhima Kiran	2015	*Rabi*	Light red, globe shaped and thin neck bulbs
7	Bhima Super	2016	*Kharif & LK*	Pink, globe shaped and thin neck bulbs
8	Bhima Safed	2018	*Kharif*	White coloured, globe shaped and thin neck bulbs
9	Bhima Light Red	2018	*Rabi*	Light red, globe shaped and thin neck bulbs
10	Bhima Shakti	2019	*LK & Rabi*	Medium red, flat-globe shaped and thin neck bulbs
11	Bhima Gulab (RGP-3)	2024	*Kharif*	Dark red colour, globe shaped bulbs
12	Bhima Kesar (DOGR-1625)	2024	*Kharif*	Dark red colour, globe shaped bulbs
13	Bhima Early (DOGR-1203-DR)	2024	*Rabi*	Dark red colour, oval shaped bulbs
14	Bhima Jyoti (DOGR-1550-Agg) (Multiplier onion)	2024	*Rabi*	Medium red colour, ovate shaped bulblets
15	Bhima Jata (DOGR-1546-Agg) (Multiplier onion)	2024	*Rabi*	Pink colour, elliptic shaped bulblets
16	DOGR-HT-3 (Bhima Prasanskaran)	2024	*Rabi*	White colour, flat globe shaped bulbs
17	DOGR-HT-4 (Bhima Nirjala)	2024	*Rabi*	White colour, flat globe shaped bulbs
18	DOGR-361 (Bhima Ujjwala)	2024	*Rabi*	White colour, globe shaped bulbs

3. Genetic Resource Base at ICAR-DOGR: Foundation for Hybrid Breeding

The successful development of onion hybrids hinges on access to a rich and diverse pool of genetic resources that underpin heterosis breeding, cytoplasmic male sterility (CMS)-based hybrid seed production, stress tolerance and quality enhancement. Genetic diversity among parental lines is critical to unlocking hybrid vigour, enabling breeders to combine complementary traits and produce hybrids with superior performance in yield, disease resistance, and environmental adaptability (Mahajan and Gupta, 2023). At the national and international levels, onion genetic resources are conserved in gene banks such as the ICAR-NBPGR (India) and the National Plant Germplasm System (NPGS, USA). ICAR-DOGR, Pune has been recognized as an active onion germplasm site and currently maintains 1,523 accessions including indigenous collections, exotic introductions, landraces and breeding lines. These accessions are characterized under field conditions for traits relevant to hybrid development, such as earliness, storability, disease resistance, bulb shape and adaptation to different agro-climatic zones (Table 2).

Table 2. Status of onion germplasm collection and maintenance at ICAR-DOGR, Pune

S. No.	Onion types	Number of accessions
1.	Onion (Dark Red)	325
2.	Onion (Light Red)	518
3.	Onion (White)	309
4.	Onion (Yellow)	35
5.	Onion (Rose Type)	9
6.	Multiplier onion	62
7.	Exotic onion	82
8.	Long day onions	94
9.	Wild species	89
	Total	**1523**

Despite the recognized economic and medicinal significance of wild *Allium* species, their representation in germplasm collections remains limited due to restricted access to their native habitats. However, efforts at ICAR-DOGR have included assessment of species diversity and prioritization of wild *Allium* conservation (Gupta et al., 2018a; Gopal et al., 2016). Wild relatives like *Allium roylei*, *A. galanthum* and *A. fistulosum* have been used in interspecific hybridization programs to introgress genes for resistance to biotic and abiotic stresses (Scholten et al., 2007; Vu et al., 2012; Kunchge et al., 2012; Gupta et al., 2015). These have opened up novel avenues for crop improvement and broadened the genetic base for hybrid breeding (Mahajan et al. 2015).

ICAR-DOGR, Pune has developed several unique genetic stocks that possess important agronomic traits and were being utilized as valuable donor parents in hybrid breeding programs. Among these, DOGR-1666 exhibits tolerance to waterlogging, while DOGR-1656 shows resilience under drought conditions. DOGR-1168 has demonstrated strong bolting tolerance, a critical trait for maintaining bulb quality in fluctuating climates. Additionally, early maturity traits have been identified in genotypes

such as DOGR-1203-DR (Gupta et al. 2014, 2015a), DOGR-1549-Agg (Gupta et al. 2018a) and DOGR-1523-Agg. These lines are being systematically introgressed into CMS and restorer backgrounds to develop onion hybrids with early maturity and adaptability to biotic and abiotic stresses.

Table 3. Unique genetic stocks of onions available at ICAR-DOGR, Pune

Sr. no	Accession	Registration Id	Characteristics
1	Bhima Early (DOGR-1203-DR)	INGR14057	Early Maturity and 100% neck fall
2	DOGR-1549-Agg*	INGR16006	Early Maturity and pink bulblets
3	DOGR-1666	INGR22082	Waterlogging tolerance
4	DOGR-1656	INGR22083	Drought tolerance
5	DOGR-1168	-	Bolting tolerance
6	DOGR-1523-Agg*	-	Early maturation and uniform bulblets numbering 5-6

*Multiplier onion

Bhima Early (DOGR-1203-DR) | DOGR-1549-Agg | DOGR-1656 | DOGR-1666

Fig. 3. Unique genetic stocks of onions available at ICAR-DOGR, Pune

4. Early Initiatives on exotic hybrids

In the initial phase of hybrid onion research, ICAR-DOGR undertaken the evaluation of exotic hybrids to explore their performance under Indian agro-climatic conditions. A total of 65 exotic hybrids were introduced from multiple international sources, including NRI (UK), Hazera Seeds Pvt. Ltd. (Israel), D. Palmer Seeds (USA), Nunhems and Pro Agro Seeds. These hybrids were evaluated over two to three years during both *late kharif* and *rabi* seasons to assess their yield potential and adaptability (Table 4).

Among the tested entries, a few hybrids Mercedes, Linda Vista, Collina, Couger, Rio Raji Red, and Cadillac demonstrated superior bulb yield under Indian conditions. Notably, Collina and Couger gained popularity among farmers and were even exported to Germany, reflecting their market acceptability and uniformity. However, despite good bulb formation, these hybrids exhibited a major limitation: failure to produce seeds in Indian conditions due to their intermediate and long-day photoperiodic requirements. As a result, seed multiplication of these hybrids in India was not feasible, making farmers dependent on private seed companies for seeds produced in long-day temperate regions. This dependency posed challenges in terms of seed cost, availability and sustainability of hybrid cultivation in India. These early efforts, while limited by photoperiod sensitivity, laid the groundwork for understanding the necessity of developing indigenous short-day onion hybrids adapted to Indian environments.

Table 4. Performance of some promising exotic hybrids during *late kharif* and *rabi* season

Late *kharif*			*Rabi*		
Year	**Line/hybrid**	**Yield (t/ha)**	**Year**	**Line/hybrid**	**Yield (t/ha)**
1998-1999	Mercedes	38.30	1998-1999	H 1367	42.40
	H 1268	38.30		Yellow Granex	35.40
	RM 771	37.50		H 1338	34.20
	Rouge de Tana	34.40		H 60	31.80
	RM 7936	31.70		Grano F_1	29.80
	H 650	65.40	1999-2000	Mercedes	40.19
	H 893	63.30		Rio Raji Red	33.81
	Ringer Grand Improved	59.80		3404 Hybrid	28.47
	Grano F_1	58.60		Hybrid-3667	18.43
1999-2000	Rio Raji Red	18.95		Santiago	22.26
	Mercedes	37.42		-	-
	Pinnacle	5.36		-	-
	Teton	16.28		-	-
	PS 1190	20.32		-	-
	Red Creole	19.14		-	-
2000-2001	Santiago	22.8	2000-2001	Twister	78.8
	Mercedes	22.9		Couger	76.8
	Twister	35.1		Cyclone	94.0
	Couger	22.0		Mercedes	77.8
	Red Creole	17.4		Red Creole	24.0
	Rio Raji Red	52.0		Linda Vista	87.8
	Cadillac	36.6		Cadillac	94.8
	Linda Vista	47.8		Santiago	69.4
	Cyclone	46.4		-	-
2001-2002	Cyclone	86.5	2001-2002	-	-
	Cadillac	65.0		-	-
	Couger	58.5		-	-
	Mercedes	47.1		-	-
	Pinnacle	44.6		-	-
	Linda Vista	44.3		-	-
2004-2005	Nineteen exotic hybrids	51.0-97.8		-	-

(DOGR 1998-2000)

5. Heterosis studies at ICAR-DOGR

During 2001, line × tester program was initiated in which two male sterile lines (MS48 A and MS 65A) were crossed with 42 selected lines. Out of these, seeds were obtained from 36 crosses with MS 48A and from 37 crosses with MS 65A and evaluated for heterosis (DOGR, 2000-2001). Crosses with MS 65A were found to be more promising as compared to crosses with MS 48A. Percentage superiority in five F_1 hybrids made between male sterile line MS 65A and the inbred lines *viz.*; 444, 14-2-W, W-153, 450 and 208 was significantly higher than the best check ALR (54.0 t/ha). None of the F_1 hybrids made between male sterile line MS 48A and the inbred lines were significantly superior to the best check ALR. It was observed that F_1's made with MS 48A showed very less heterosis as compared with the F_1's made with MS 65A (DOGR, 2001-02). Similarly, crosses made in 2004-05, cross with MS 48A gave significant heterosis over better parent (54.5%). For marketable yield 21 to 50% heterosis over check was observed. Heterosis over better parent was exceeded up to 60 % and 70% during *kharif* and *rabi* 2021-22 (Table 5). Heterosis for high yield, earliness and other agronomic characteristics was noticed by Gupta et al. (2011) in onion hybrids. These findings emphasize the potential of heterosis in boosting onion productivity and the effectiveness of hybrid breeding strategies. Onion crosses evaluated from 2003 to 2022 demonstrated remarkable yield improvements across seasons. Several crosses consistently outperformed the best checks, with standard heterosis % (SH) values ranging widely-from modest gains (~15%) to exceptionally high levels exceeding 130%. The highest SH was recorded during *rabi* 2003-04 (136.40%), followed by substantial SH in both *kharif* and Late *Kharif* in subsequent years. The *Rabi* season generally exhibited higher SH due to favourable growing conditions, but significant heterosis was also observed in Late *Kharif* (up to 88.94%) and *Kharif* (up to 112.02%) seasons in later years (Table 5).

Table 5. Evaluation of crosses with *MS* lines during *kharif*, late *kharif* and *rabi* over the years at ICAR-DOGR

Year	No. of crosses	Superior crosses with MY (t/ha)	Season	Best Check	SH (%)
2003-04	80	5 (24.20-39.20 t/ha)	*Rabi*	ALR (16.60 t/ha)	136.40
2004-05	4	4 (27.10-31.80 t/ha)	*Rabi*	N-2-4-1 (23.44 t/ha)	18.89-28.29
2007-08	11	Nil	*Kharif*	Bhima Super (24.80 t/ha)	Nil
	17	4 (30.40-35.50 t/ha)	*Rabi*	N-2-4-1 (28.10 t/ha)	34.50
2009-10	3	1 (21.60 t/ha)	*Kharif*	Bhima Super (14.70 t/ha)	46.70
2010-11	40	4 (8.89- 49.28 t/ha)	*Rabi*	N-2-4-1 (39.84 t/ha)	> 10
	30	4 (8.33- 38.33 t/ha)	*Kharif*	Bhima Super (22.06 t/ha)	> 35
2011-12	22	4	*LK*	Bhima Super (25.56 t/ha)	21-50
	53	3	*Kharif*	Arka Lalima (25.40 t/ha)	26.80
	55	6 (>30 t/ha)	*Rabi*	Bhima Kiran (32.60 t/ha)	21-34
	10*	1 (44 t/ha)		Phule Safed	28.14

Year	No. of crosses	Superior crosses with MY (t/ha)	Season	Best Check	SH (%)
2012-13	58	10	*Kharif*	Arka Lalima (20.61 t/ha)	41.53
	33	4	*LK*	Bhima Shakti (31.62 t/ha)	>20
	60	6	*Rabi*	Bhima Kiran (25.30 t/ha)	47.12
	12*	3 (31.19-42.90 t/ha)		Phule Safed	15.52-29.15
2013-14	25	2	*LK*	Bhima Shakti (45.08 t/ha)	15.84-21.38
	7*	2 (34.79-35.56 t/ha)	*Rabi*	Bhima Shweta (32.22 t/ha)	28.70-69.30
2014-15	34	3	*LK*	Bhima Shakti (43.04 t/ha)	> 15
	57	4	*Rabi*	Bhima Kiran (32.31 t/ha)	> 15
	59	10	*Kharif*	Bhima Super (29.12 t/ha)	> 30
	13*	1 (37.8 t/ha)	*Rabi*	Bhima Shweta	
2015-16	60	4 (24.01-32.68 t/ha)	*Kharif*	BDR (24.01 t/ha)	13.34-36.11
	59	5 (46.47-53.79 t/ha)	*LK*	Bhima Super (38.45 t/ha)	20.85-39.89
	60	3 (56.37-59.09 t/ha)	*Rabi*	Bhima Shakti (48.76 t/ha)	15.61-21.18
2016-17	110	5 (67.93-86.80 t/ha)	*Kharif*	BDR (58.71 t/ha)	15.71-30.81
	5*	2 (35.27-45.91 t/ha)		Bhima Shubhra (32.76 t/ha)	22.29-40.13
	57	4 (45.83-52.93 t/ha)	*LK*	Bhima Super (39.77 t/ha)	15.21-33.10
	58	4 (28.80-36.21 t/ha)	*Rabi*	Bhima Shakti (24.05 t/ha)	19.74-50.54
	16*	5 (28.39-43.11 t/ha)		Bhima Shweta (27.11 t/ha)	24.86-79.16
2017-18	110	10	*Kharif*	Bhima Super (19.76 t/ha)	>25
	10*	4 (14.91-20.70 t/ha)		Bhima Shubhra (12.92 t/ha)	18.14-69.33
	33	3 (50.69-71.33 t/ha)	*LK*	Bhima Shakti (42.89 t/ha)	18.19-66.31
	8*	1 (45.10 t/ha)		Bhima Shubhra (35.60 t/ha)	22.10
	97	5 (29-38.33 t/ha)	*Rabi*	Bhima Kiran (20.29 t/ha)	42.93-88.94
2019-20	63	5 (18.56-27.33 t/ha)	*Kharif*	Bhima Super (28.54 t/ha)	43.99-112.02
	105	7 (54.22-69.33 t/ha)	*LK*	Bhima Shakti (44.34 t/ha)	22.28-56.36
	104	7 (56.67-60.67 t/ha)	*Rabi*	Bhima Kiran (45.31 t/ha)	25.07-33.89
2020-21	50	5 (57.33-65.83 t/ha)	*LK*	BSS-441 (43.83 t/ha)	30.80-50.19
	71	7 (54.27-61.87 t/ha)	*Rabi*	Bhima Kiran (38.75 t/ha	40.03-59.64
	13*	3 (43.44-50.87 t/ha)		Bhima Shweta (32.43 t/ha)	34.11-58.92

Year	No. of crosses	Superior crosses with MY (t/ha)	Season	Best Check	SH (%)
2021-22	22	5 (29.76-42.27 t/ha)	*Kharif*	BDR (25.35 t/ha)	17.40-66.75
	60	5 (39.25-56.89 t/ha)	*LK*	Bhima Shakti (32.93 t/ha)	19.19-72.76
	93	5 (41.87-52.00 t/ha)	*Rabi*	Bhima Shakti (28.68 t/ha)	45.98-81.32
	15*	4 (54.06-67.71 t/ha)		Bhima Shweta (40.81 t/ha)	32.47-65.54
2023-24	65	4	*Kharif*	Bhima Super (32.98 t/ha)	>91
	61	5	*LK*	Bhima Shakti (38.15 t/ha)	>51
	47	5	*Rabi*	Bhima Shakti (31.94 t/ha)	>13

*White onion hybrid crosses; SH- Standard Heterosis (%)

6. Milestone projects fueling on Hybrid Onion Development

The foundation for hybrid onion breeding was laid under the NATP initiated in 2004 at ICAR-DOGR. This program marked the first national-level platform where hybrid development was taken up systematically across multiple locations. Building upon NATP's, the AINRPOG was launched in 2008-09 to unify and coordinate onion and garlic research at 28 centers across the country. ICAR-DOGR acts as the coordinating centre for this project, managing 10 main, 13 voluntary and 5 cooperating centers across the country. These centers rigorously test pre-released hybrids and open-pollinated varieties under different seasons (*kharif*, late *kharif* and *rabi*), allowing for region-specific recommendations and performance validation.

As exotic hybrids failed to produce seeds under short day tropical conditions the next focus was given to identification and development of male sterile lines in Indigenous onion genotypes. Meanwhile some local Indian cultivars were reported to possess male sterility such as N 2-4-1 (Patil et al. 1973), Nasik White Globe (Pathak et al. 1980) and Bombay White Globe (Pathak et al. 1986) and Pusa Red, IIHR-20 (DARE, 1986). Using these sources two male sterile lines were developed MS 48A and MS 65A and used to develop two hybrids at IIHR, Bengaluru. These male sterile lines along with their maintainer lines were procured to ICAR-DOGR in 1999. Subsequently these lines were used to produce several crosses with inbred lines to obtain the best performing hybrids. Initially 73 crosses were made using these two MS lines. Crosses with MS 65A were found to be more promising as compared to crosses with MS 48A. Storage studies also revealed poor storage potential of MS 48 A and its crosses. It was also observed that F_1's made with MS 48A showed very less heterosis as compared with the F_1's made with MS 65A. Further, microscopic studies of pollen viability revealed MS 48A 60% fertile while MS 65 A 40% fertile.

Recognizing the need to integrate public and private expertise, ICAR-DOGR initiated a Joint Venture in 2008 with Bejo Sheetal Seeds. This project aimed at acquiring elite CMS lines (MS 111A, MS 222A, MS 1600A for red onion and MS 100A for white onion) enlisted in Table 6, strengthening hybrid seed production and identifying and validating stable CMS systems in Indian agro-climatic conditions. Initially 20 crosses were made using MS 888A and MS 999A were made during 2008-09. However,

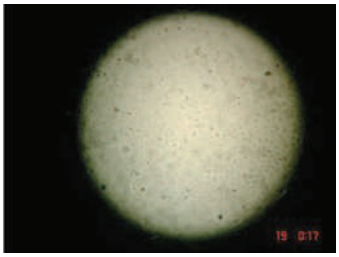
due to observation of doubles and colour variation in these MS lines, these were discontinued. Further, 4 MS lines were procured and crossed with pollinators at ICAR-DOGR producing 40 crosses during 2009-10. In 2011, 48 new combinations were made out of these 48 combinations, eight F_1 hybrids including six red (DOGR Hy-3, DOGR Hy-4, DOGR Hy-5, DOGR Hy-7, DOGR Hy-8 and DOGR Hy-50) and two white hybrids (DOGR WHY-1 and DOGR WHY-2) introduced in AINRPOG in 2016. In later phase, 110 hybrid combinations were made, out of which seven red onion F_1 hybrids (DOGR Hy-155, DOGR Hy-156, DOGR Hy-172, DOGR Hy-56, DOGR Hy-207, DOGR Hy-211 and DOGR Hy-212) have been introduced in AINRPOG in 2019 (Project report, 2019). The detailed list of hybrids introduced in AINRPOG has been given in Table 9.

Male sterile line Development

In 2014, Flagship Programme on Studies on Male Sterility and Development of Hybrids in Onion (2014-2017) was initiated primarily focusing on identification of CMS sources in Indian germplasm, marker-assisted screening of genotypes for cytoplasm types. Under this project to identify male sterile cytoplasm, population of 30 genotypes were screened using markers specific to mitochondrial rearrangements at sites near to *orf501* and *5′-cob* gene, which was responsible for male sterility. Among them 4 genotypes exhibited presence of mitochondrial rearrangement for S type cytoplasm and three for T type cytoplasm. Commercially available hybrids were evaluated for type of cytoplasm and amplification of two fragments (414bp, 180bp) from *cob* specific primers found to have S type cytoplasm. This confirms that hybrids such as BSS-442, BSS-226, BSS-133, BSS-255, BSS-230, and Reforma possess S-type cytoplasm, which was associated with male sterility. These findings validate the successful incorporation of CMS lines in these hybrids (Table 7). Further in 2015-16, 5000 plants of red onion germplasm were screened for naturally occurring male sterile plants. Male sterile plants were observed in RGP-4-Sel and Arka Kalyan. Pollens of these two naturally occurred new male sterile lines were also confirmed for sterility through acetocarmine test (Fig. 6) (Gupta et al. 2016). Further characterization by Khar et al. 2022, Arka Kalyan reported to have 5 sterile plants of S-cytoplasm type out of 8 plants. To identify the male-fertility restoration locus (Ms), 35 breeding lines were screened for Ms locus specific markers. Genotyping with PCR-based markers proved effective for the marker-assisted selection (MAS) of the nuclear male-fertility locus in onion breeding lines. The *jnurf20* marker, due to its dominant nature, was able to identify homozygous recessive lines but could not distinguish heterozygous individuals from homozygous dominants, limiting its utility in differentiating all genotypic classes. In contrast, the *PsaO* marker, being co-dominant, successfully differentiated all allelic variants at the Ms locus across breeding lines. Despite some limitations in marker coverage across diverse genetic backgrounds, *PsaO* remains a robust and powerful tool for MAS, particularly in segregating populations. Collectively, both markers serve as valuable resources in onion breeding programs targeting the efficient selection of male sterility and fertility restoration traits (Fig. 7) (DOGR, 2020). This will help to identify the naturally occurring male sterile cytoplasm which can be exploited for generation of male sterile lines adapted to local condition (DOGR, 2012-13). Moreover, molecular characterization for cytoplasm types for further confirmation is necessary.

Male sterile RGP-4-Sel

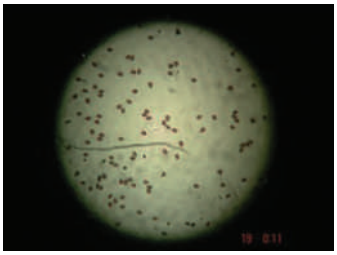
Male fertile RGP-4-Sel



Male sterile pollens of RGP-4-Sel

Male sterile flowers of Arka Kalyan

Male fertile flowers of Arka Kalyan



Male fertile pollens of RGP-4-Sel

Fig. 4. Male sterile plants identified in RGP-4-Sel and Arka Kalyan

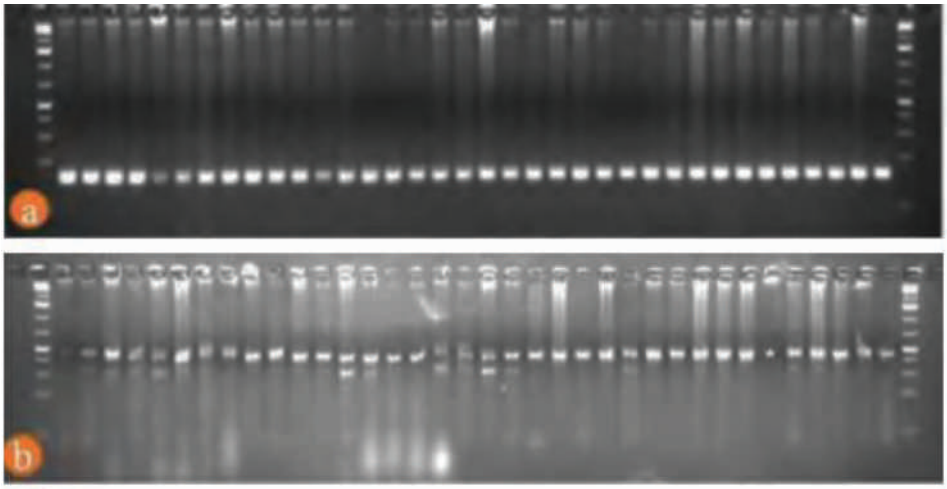


Fig. 5. Marker assisted selection of *Ms* locus among breeding lines, a) *jnurf* 20 marker genotyping for *Ms* locus, b) *PsaO* gene markers genotypes for Ms loci, (1Kb plus DNA ladder used for comparison of amplicon fragments

Table 6. Male sterile lines available at ICAR-DOGR

***MS* lines**	**Features**	**Source**
MS 48A	Dark red onions, flat globe shape, tolerant to purple blotch and medium sized bulbs	IIHR, Bengaluru
MS 65A	Light red coloured, oval-globe shaped, firm bulbs, medium sized, good keeping quality	IIHR, Bengaluru
MS 111A	Suitable for *rabi*, light red bulbs	ICAR-DOGR
MS 222A	Suitable for *rabi*, medium red bulbs	ICAR-DOGR
MS 1600A	Suitable for *kharif*, dark red bulbs	ICAR-DOGR
MS 100A	Suitable for *rabi*, white bulbs	ICAR-DOGR

Table 7. Screening for male sterile cytoplasm in hybrids available in India

Sr. No	**Variety Name**	**473 bp (*orf501*)**	**414 bp (*5'-cob*)**	**Cytoplasm**
1	BSS-442	+	+	S
2	BSS-226	+	+	S
3	BSS-133	+	+	S
4	BSS-255	+	+	S
5	BSS-230	+	+	S
6	Reforma	+	+	S

Three MS lines were crossed with each of three selected elite lines to transfer male sterility in improved varietal backgrounds as per procedure suggested by Pike, (1986). About 100 plants of each MS lines were selected at random and crossed with selected male parent. Simultaneously, half of the umbels of selected plants of male parent in each cross were selfed for its B line development. About 50% selected male sterile plants produced F_1 seeds along with self-seed of male plants. F_1 progenies with 100% male sterile plants indicate which selections are B lines. Seeds of the selfed selection from each test cross were saved so that once the B lines are identified; they can be used in backcross programme to develop A line (Gupta et al. 2015b) (Fig. 8). Currently, ICAR-DOGR possesses 5 MS lines of red onion and one MS line of white onion. Male sterile plants have been also identified in Arka Kalyan and RGP-4-Sel described under section 5.3.

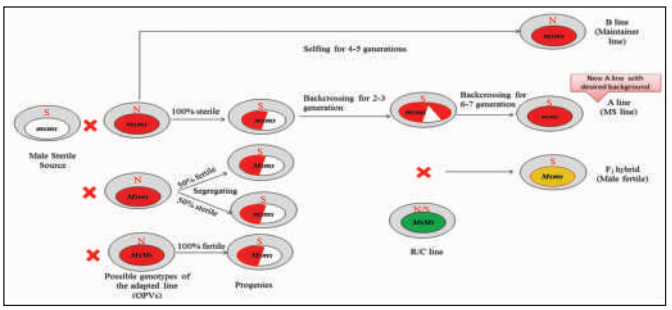


Fig. 6. Procedure followed at ICAR-DOGR for development of new MS lines in adapted background of genotypes

Fig. 7. Male sterile lines available at ICAR-DOGR

For development of cytoplasmic lines through targeted mutagenesis a project on "Development of cytoplasmic male sterile lines in onion through targeted mutagenesis of *AcMSH1* gene" was launched during 2022. CRISPR/Cas9-mediated gene editing of the *AcMSH1* gene in onion (cv. B. Super) using *Agrobacterium*-mediated transformation was started in 2022. Two CRISPR constructs targeting exon 2 and exon 3 were transformed into Agrobacterium strain LBA4404 and used for onion calli transformation. After selection on Hygromycin B, transformed calli were screened and transferred to shooting media. Out of 11 transformation batches, 1 batch per construct reached the shooting stage, 7 batches were still in selection, and 2 were in the resting phase. Additionally, an ELISA-based immunoassay was performed to determine the subcellular localization of *AcMSH1* protein. Protein was isolated from mitochondria, chloroplasts, and their fractions. Results showed that *AcMSH1* protein was predominantly localized in mitochondria, followed by chloroplasts, with negligible amounts in organelle fractions (DOGR, 2022).

The high expression of *AcMSH1* in shoot tips underscores its likely role in regulating organogenesis or genome stability in dividing tissues. Conversely, its low expression in embryogenic calli could favour transformation processes, potentially making it a suitable target for genome editing approaches aimed at inducing variation or haploid induction (Fig. 10). These findings could inform strategies for manipulating *AcMSH1* activity to optimize tissue culture and transformation protocols in onion breeding programs.

Consortium Research Platform (CRP) on Hybrid Technology in Onion (2024) was initiated at ICAR-DOGR Pune, ICAR-IARI New Delhi and ICAR-VPKAS Almora in March 2024 to develop stable, high yielding F_1 hybrids through male sterile lines. Five male sterile lines *viz.*, MS 48A, MS 65A, MS 111A, MS 222A and MS 1600A along with inbred lines are being utilized to develop stable F_1 hybrids. A total of 70 crosses were made using these male sterile lines, inbred/elite lines and will be evaluated in

next season. Also 26 F_1 hybrids were evaluated including their parents and three standard check varieties. In addition, several crosses were made between DOGR-1043, 1044, 1606, 1609 and 1613 as well as between DOGR-1611, 1626, 1672, 1757, 1758, 1647, 1653 and 1636 with DOGR 1203-DR, 1669 and 1168. Nine varieties, Bhima Kiran, Bhima Shakti, Bhima Raj, Bhima Super, Bhima Red, BLR, BDR, RGP-3 and DOGR-1625 were crossed with DOGR 1203-DR with the goal to transfer earliness trait in the progeny of these crosses, which can be evaluated for early maturity. In this project, new male sterile sources will be screened using molecular markers.

Inbred lines development

Along with male sterile (MS) lines, inbred lines are crucial for the development of onion hybrids. These hybrids are derived from genetically diverse parental lines, with inbred lines providing the necessary genetic contrast to enhance heterosis. Well-developed inbreds contribute significantly to improve yield, disease resistance, and other agronomic traits in the resulting hybrids. However, the development of inbred lines in onion poses a major challenge due to severe inbreeding depression observed in successive generations upon selfing (Gupta and Singh, 2016).

Despite these challenges, efforts are ongoing at ICAR-DOGR to develop stable inbred lines. Initially, twenty inbred lines namely 271, 283, 546, 553, 571, 592, 593, 594, 595, 597, 600, 614, 635, 644, 646, 650, 651, 657, 670, and 671 were developed during *rabi* 1999-2000 based on key horticultural traits. These lines were subsequently planted in the *late kharif* season of 2000 to obtain the first generation of inbreds.

As of the 2023-24 assessment, ICAR-DOGR has advanced a diverse set of inbred lines at various stages: 36 lines in the I_1 generation, 68 lines in I_2, 16 lines in I_3, and 6 lines in the I_4 stage (Table 8) (DOGR, 2023-24).

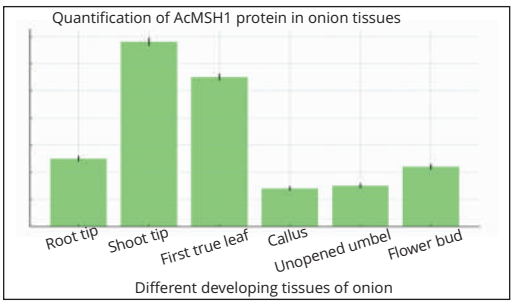


Fig. 8: Quantification of AcMSH1 protein in different developing tissues of onion

Fig. 9. Development of inbred lines in onion at ICAR-DOGR

Table 8. Inbreds developed at ICAR-DOGR

Stage	Inbreds
I_1 (36)	1605, 1606, 1613, 1617, 1621, 1625, 1632, 1633, 1634, 1636, 1644, 1648, 1654, 1657, 1667, 1669, 1670, 1694, 1725, 1730, 1750, 1754, 1809, C6-KM-2, DOGR-1172, DOGR-571-LR, EL-1751, KH-M-1, KH-M-3, LK-07-C2/DR-4, LK-07-C2/DR-3, RGP-1, RGP-2-LK-Sel, RGP-4, R-KH-M-II and R-Rb-M-IV
I_2 (68)	1222, 1224, 1231, 1241, 1246, 1250, 1252, 1281-1, 1371, 1414, 1449, 1604, 1606, 1612, 1614, 1617, 1618, 1623, 1625, 1627, 1632, 1635, 1636, 1638, 1642, 1646, 1648, 1655, 1657, 1659, 1660, 1661, 1664, 1667, 1669, 1670, 1694, 1724, 1725, 1729, 1737, 1743, 1746, 1750, 1751, 1754, 1757, 1764, 1767, 1769, 1774, 1782, 1784, 1785, 1786, 1787, 1809, 571-LR, C6-KM-2, DOGR-1044-Sel, DOGR-1172-DR, LK-07-C2/LR-3, Red Genepool-4, RGP-1-Kh-Sel, RGP-4, R-KH-M-3, R-KH-M-I and R-LK-M-I
I_3 (16)	1241-1, 1371-2, 1450-1-1, 1481, 1613, 1629, 1632, 1636-2, 1649-1, 1665, 1665-1, DOGR-1048-Sel, DOGR-651-Sel, DOGR-670-Sel, R-Rb-M-II and R-Rb-M-III
I_4 (6)	1636, 1055-1, 1668-3, 1065, 1665-1 and 788

7. Backcrossing for trait transfer

To enhance earliness in onion, crosses were initiated between the early maturing line DOGR 1203-DR and elite lines. In 2013-14, eighteen such crosses were made and evaluated during the *rabi* season. These crosses demonstrated promising performance for uniform neck-fall and early maturity. Notably, eight F_3 populations derived from crosses involving three elite lines recorded marketable yields exceeding 60 t/ha. In parallel, ICAR-DOGR has identified unique onion germplasm exhibiting tolerance to both waterlogging and drought stress. These resilient genotypes present valuable genetic resources for incorporating abiotic stress tolerance into hybrids, thus aiding the development of climate-resilient onion cultivars (Gedam et al., 2021; 2022).

Further, significant progress has been made in improving short-day onions through introgression of genes from long-day genotypes. A total of 135 crosses were executed by combining available exotic long-day hybrids with short-day varieties. Progenies from these crosses were selected based on key bulb characteristics including colour, shape, size, and storability. Evaluation and selection were carried out jointly by ICAR-DOGR, Pune and ICAR-CITH, Srinagar. Selected bulbs from each cross were subsequently sent to ICAR-CITH for seed production. Currently, 18 sixth-generation populations are under evaluation under short-day conditions. Among these, crosses such as A-1 × H-8, A-1 × M-13, Q-17 × K-11, and U-21 × M-13 are noteworthy for their superior bulb yield and improved storability under short-day agro-climatic zones (DOGR, 2022).

Efforts are also underway to transfer male sterility (MS) into red onion varietal backgrounds through backcrossing. Red onion varieties were crossed with MS lines, and the bulbs obtained from backcross progenies were used for further backcrossing to introgress MS genes into elite red varieties. Currently, six combinations are in the BC_1 stage, and three combinations have advanced to the BC_3 stage. These

efforts target the integration of MS into popular DOGR varieties such as Bhima Super, Bhima Dark Red, Bhima Kiran, Bhima Shakti and DOGR-1133 (Fig. 8).

8. Promising F_1 hybrids developed by ICAR-DOGR

Over the years, extensive hybridization and evaluation programs at ICAR-DOGR have led to the development of numerous promising onion hybrids. Initial small-plot evaluations of several crosses yielded up to 50 t/ha, significantly outperforming standard checks (15-20 t/ha). In 2004, under the National Agricultural Technology Project (NATP), systematic hybrid evaluations were initiated. F_1 hybrids developed using four CMS lines under the hybrid network program exhibited high heterosis, ranging from 17 to 59% over the standard check 'N 2-4-1' and 11 to 50% over 'ALR'. During *rabi* evaluation, five F_1 hybrids DOGR Hy-50, DOGR Hy-2, DOGR Hy-5, DOGR Hy-7, and DOGR Hy-8 showed more than 30% heterosis in marketable yield over the standard check Bhima Kiran (25.3 t/ha) (Gupta et al. 2018b). Similarly, 10 hybrids showed more than 20% heterosis during *kharif* over Arka Lalima (20.6 t/ha) (Veere Gowda and Gupta, 2014; Gupta et al. 2017). Hybridity of nine hybrids including DOGR Hy-1 to DOGR Hy-8 and DOGR Hy-50 has been confirmed using SSR markers (Gupta et al. 2020).

From 2000 to 2023, over 200 hybrid combinations were evaluated, resulting in the identification of more than 15 promising hybrids, which were subsequently tested for three years under AINRPOG multi-location trials during *kharif* and *rabi* seasons. Notably, seven of these hybrids consistently yielded above the national average, with DOGR Hy-8 recording the highest productivity of 54.5 t/ha. These hybrids also demonstrated zero bolting and moderate to high bulb storability, strengthening their candidacy for wider cultivation. Early maturing hybrids such as DOGR Hy-4, Hy-7 and Hy-73 are particularly suited for short-season growing environments (Table 9).

To further diversify the hybrid base, 110 F_1 hybrids of red onion were developed using five CMS lines (MS 48A, MS 65A, MS 111A, MS 222A, and MS 1600A) crossed with 22 elite pollinators lines including 546-DR, 571-LR, KH-M-1, KH-M-2, RGP-1, RGP-2, RGP-3, RGP-4, RGP-5, 1604, 1605, 1606, 1607, 1608, 1609, 1612, 1613, 1629, 1630, 1657, 1663 and 1666. These hybrids and their parental lines were evaluated across *kharif*, late *kharif*, and *rabi* seasons. Six hybrids DOGR Hy-6, Hy-73, Hy-156, Hy-172, Hy-173, and Hy-179 emerged as promising and were advanced to AINRPOG trials for multi-location assessment. All exhibited have good storability, early maturity, and were free from bolters and doubles. Among these, DOGR Hy-156 and Hy-179 were particularly noteworthy.

Table 9. Performance of DOGR F_1 hybrids entered in AINRPOG trials

Entry	MY (t/ha)	SH (%)	ABW (g)	Bolter (%)	DTH	Season	Storability	Bulb Shape	Bulb Colour
DOGR Hy-1	41.30	42.84	68.20	0.0	103	Rb	Good	Flat Globe	LR
DOGR Hy-2	34.96	20.91	64.50	0.0	100	Rb	Good	Globe	DR
DOGR Hy-3	34.20	16.22	68.95	0.0	95	Kh	Medium	Globe	MR

Entry	MY (t/ha)	SH (%)	ABW (g)	Bolter (%)	DTH	Season	Storability	Bulb Shape	Bulb Colour
DOGR Hy-4	34.08	15.81	80.21	0.0	94	Kh	Medium	Flat Globe	DR
DOGR Hy-5	38.73	20.11	75.21	0.0	101	Kh	Medium	Globe	DR
DOGR Hy-6	41.86	17.87	70.00	0.0	108	Rb	Good	Flat Globe	MR
DOGR Hy-7	40.75	34.88	68.72	0.0	105	Kh & Rb	Very good	Flat Globe	DR
DOGR Hy-8	54.45	23.57	83.33	3.89	118	LK	Good	Flat Globe	MR
DOGR Hy-50	37.45	24.11	63.44	0.0	106	Rh	Very good	Globe	DR
DOGR Hy-156	43.12	18.95	87.00	0.0	108	Rb	Good	Globe	LR
DOGR Hy-172	47.67	17.90	72.00	0.0	108	Rb	Good	Globe	MR
DOGR Hy-73	33.67	11.20	61.30	0.0	91	Kh	Good	Globe	DR
DOGR Hy-173	40.55	30.59	91.00	0.0	111	Rb	Good	Globe	MR
DOGR Hy-179	36.17	16.48	60.80	0.0	108	Rb	Good	Globe	MR

Among these promising hybrids, DOGR Hy-156 and DOGR Hy-173 have demonstrated significant yield advantages. DOGR Hy-156, a F_1 hybrid suitable for the *rabi* season, produces globe-shaped, light-red bulbs with uniformity and free from doubles and bolters. It recorded a marketable yield of 43.12 t/ha, which is 18.95% higher than the best check, Bhima Shakti (36.25 t/ha). The bulbs have an average weight of 87.0 g, a thin neck, and were harvested in 108 days after transplanting. They also exhibit good storability. Similarly, DOGR Hy-173, a *rabi* season F_1 hybrid, produces globe-shaped, medium-red bulbs with uniformity and free from doubles and bolters. Based on two years of data, it achieved a marketable yield of 40.55 t/ha, which was 30.59% higher than the best check, Bhima Kiran (31.05 t/ha). The bulbs have an average weight of 91.0 g, a thin neck and were harvested in 111 days after transplanting, exhibiting good storage potential.

Ten onion hybrids along with their seven parental lines were also evaluated for hybrid purity using molecular markers. Four out of ten microsatellite (SSRs- simple sequence repeats) markers were found to be polymorphic. All four polymorphic markers revealed polymorphism between the male and female parents of one or more hybrids. The markers ACM078, ACM033, and ACM180 have been identified as the three diagnostic markers for testing the genetic purity of all the ten onion hybrids *viz.,* DOGR Hy-1, DOGR Hy-2, DOGR Hy-3, DOGR Hy-4, DOGR Hy-5, DOGR Hy-6, DOGR Hy-7, DOGR Hy-8, DOGR Hy-50 and DOGR Hy-56 (Gupta et al. 2020).

To date, ICAR-DOGR has successfully developed several stable, high-yielding F_1 hybrids through the strategic use of male sterile lines. The key characteristics of these hybrids are summarized below (Table 10 & Fig. 12).

Table 10: Promising F_1 hybrids developed at ICAR-DOGR

Year	Hybrids	Characteristics
2012-13	DOGR Hy-1	DOGR Hy-1 is suitable for *rabi* season and its bulbs are light red and flat-globe in shape.
	DOGR Hy-2	DOGR Hy-2 is suitable for *rabi* season and its bulb is dark red and globe in shape.
2013-14	DOGR Hy-7	Suitable for *rabi* season and its bulbs are flat-globe and dark red. It produces uniform bulbs without doubles and bolters. The average bulb weight was 68.72 g with thin neck. Bulbs were harvested in 105 days after transplanting and storage of bulbs was very good.
	DOGR Hy-50	Suitable for *rabi* and *kharif* season and its bulbs are uniform, globe shape and attractive dark red. It is free from doubles and bolters. The average bulb weight was 63.44 g with thin neck. This hybrid is early in maturity and harvested in 106 days after transplanting, and storage of bulbs was very good.
2014-15	DOGR Hy-3	Suitable for *kharif* season and its bulbs are globe with medium red. It produced uniform bulbs, free from doubles and bolters. On the basis of two-year data, this hybrid produced 34.20 t/ha marketable yield which is 16.22% higher than best check Bhima Super (29.42 t/ha). The average bulb weight 68.95 g with thin neck. Its bulbs harvested in 95 days after transplant.
	DOGR Hy-4	Suitable for *kharif* season and its bulbs are uniform, flat-globe with attractive dark red. On the basis of two-year data, this hybrid produced 34.08 t/ha marketable yield which is 15.81% higher than best check Bhima Super (29.42 t/ha). It is free from double bulbs and bolters. The average bulb weight 80.21 g with thin neck. This hybrid is early in maturity and harvested in 94 days after transplanting.
2015-16	DOGR Hy-5	Suitable for *kharif* season having uniform, globe and dark red bulbs with thin neck. It is free of doubles and bolters. The average bulb weight was 75.21 g. This hybrid is early in maturity and harvested in 101 days after transplanting.
2016-17	DOGR Hy-8	Suitable for late *kharif* season and its bulbs are flat-globe with medium red. It produced uniform bulbs and free from doubles and bolters. The average bulb weight was 83.33 g with thin neck. These bulbs can be harvested 118 days after transplant and good in storage.
2017-18	DOGR Hy-6	Suitable for *rabi* season and its bulbs are flat-globe with medium red. It produced uniform bulbs and free from doubles and bolters. The average bulb weight is 70.0 g with a thin neck. Its bulbs harvested in 108 days after transplant and good in storage.

Year	Hybrids	Characteristics
2018-19	DOGR Hy-73	Suitable for *kharif* season and its bulbs are uniform, globe with attractive dark red. It is free from double bulbs and bolters. The average bulb weight is 61.3 g with thin neck. This hybrid is early in maturity and harvested in 91 days after transplanting.
	DOGR Hy-173	Suitable for *rabi* season and its bulbs are globe with medium red. It produced uniform bulbs and free from doubles and bolters. On the basis of two-year data, this hybrid produced 40.55 t/ha marketable yield which is 30.59% higher than best check Bhima Kiran (31.05 t/ha). The average bulb weight is 91.0 g with thin neck. Its bulbs harvested in 111 days after transplant and good in storage.
	DOGR Hy-179	Suitable for *rabi* season and its bulbs are medium red with globe shape. It produced uniform bulbs and free from doubles and bolters. The average bulb weight is 60.8 g with thin neck. Bulbs are harvested in 108 days after transplanting and good in storage.
2020-21	DOGR Hy-156	Suitable for *rabi* season and its bulbs are globe with light red. It produced uniform bulbs and free from doubles and bolters. This hybrid produced 43.12 t/ha marketable yield which is 18.95% higher than best check Bhima Shakti (36.25 t/ha). The average bulb weight 87.0 g with thin neck. Its bulbs harvested in 108 days after transplant and good in storage.
	DOGR Hy-172	Suitable for *rabi* season and its bulbs are globe with medium red. It produced uniform bulbs and free from doubles and bolters. This hybrid produced 47.67 t/ha marketable yield which is 17.90% higher than best check Bhima Shakti (40.43 t/ha). The average bulb weight 72.0 g with thin neck. Its bulbs harvested in 108 days after transplant and good in storage.
2022	DOGR Hy-56	Suitable for *rabi* season and its bulbs are flat-globe with light red.
	DOGR Hy-155	Suitable for *rabi* season and its bulbs are flat-globe with medium red.
2023	DOGR Hy-207	Suitable for *kharif* and *rabi* season and its bulbs are globe with medium red.
	DOGR Hy-211	Suitable for *rabi* season and its bulbs are globe with medium red.
	DOGR Hy-212	Suitable for *kharif* season and its bulbs are globe with dark red.
2024	DOGR Hy-169	Suitable for *kharif* season and its bulbs are globe with attractive dark red.
	DOGR Hy-202	Suitable for *rabi* and *kharif* season and its bulbs are globe with light red.

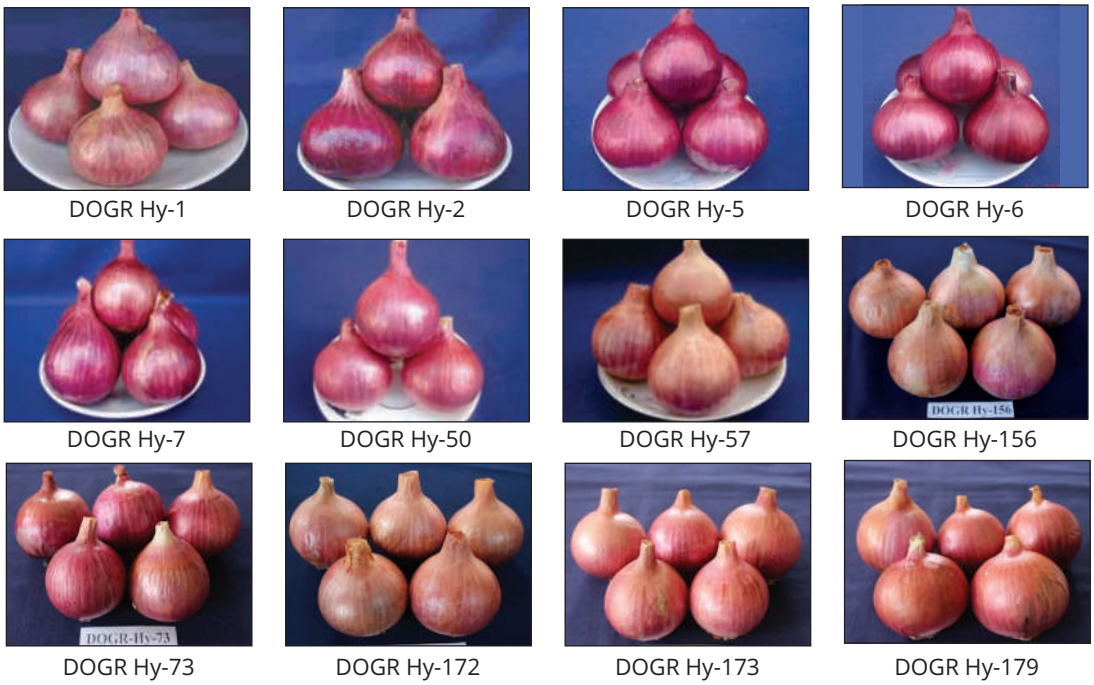


Fig. 10. Promising hybrids developed by ICAR-DOGR

9. Biotechnological advancements at ICAR-DOGR

Conventional heterosis breeding program demands availability of highly homozygous inbred lines to make pure heterotic combinations. Development of highly homozygous lines is very difficult in strictly cross-pollinated crop like onion. Efforts have been also directed toward developing inbred lines through haploid production technology at ICAR-DOGR. Haploid work was initiated in 2005-06.

Attempts were made at ICAR-DOGR in developing a doubled haploids (DH) protocol in onion, primarily through *in-vitro* gynogenesis and parthenogenesis. Initial attempts using various hormonal combinations (especially BA and BA+IAA) showed limited success, with only a few haploid plants being induced from flower bud and ovary cultures of genotypes like NHS, N-53, and N-2-4-1. Over time, the use of unopened flower buds as explants emerged as the most reliable method for gynogenesis. Experiments confirmed the importance of genotypic response, media strength, sucrose concentration (5% optimal), and hormonal combinations (BA, 2,4-D, IAA) in enhancing induction rates. Ploidy analysis using PA II (Partec, Germany) verified successful induction of haploids (72.5%), dihaploids (17.5%), and mixoploids (10%). Efforts for chromosome doubling using colchicine were initiated, and a protocol was standardized using solid MS media and 20 µM colchicine for 24-72 hrs, with high plant survival. However, success with pure male sterile (MS) lines remained limited. Parthenogenetic approaches using foreign/dead pollen yielded seeds, but most progenies were diploid and showed poor survival due to diseases and possible colchicine toxicity

(DOGR, 2005-2011).

Flower bud culture on induction media with high sucrose levels (notably 10%) showed promise, though overall gynogenic efficiency remained low (<5%), particularly when compared to long-day varieties. Different varieties exhibited variable response, with Bhima Super and N-2-4-1 showing relatively higher gynogenic potential. Gynogenic regenerants were established on MS media, with liquid cultures improving root development for cytological analysis. Ploidy analysis confirmed several true haploids, and mixoploids and diploids were also observed. Subsequent treatment with colchicine successfully induced doubled haploids, confirmed by cytology, flow cytometry, and SSR marker analysis (Fig. 11) (DOGR 2011-2014).

In 2014-15, gynogenesis was successfully induced in five common onion varieties and one multiplier type, resulting in 250 regenerants, of which 14 were confirmed as haploids through flow cytometry. Colchicine treatment (0.25 mM for 24 h) of haploid plants led to 9 doubled haploids, with three lines progressing to field planting for bulb production. Notably, one DH line from Bhima Kiran displayed tillering like a multiplier type, suggesting possible somaclonal variation or trait introgression.

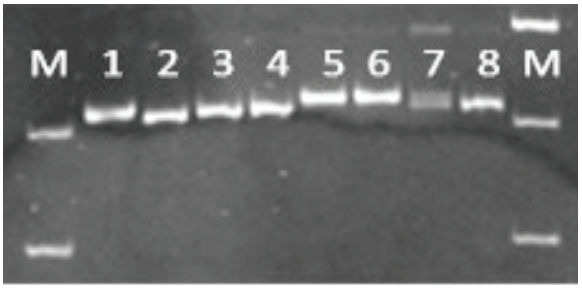


Fig. 11. SSR analysis showing homozygosity in gynogenic diploids

In 2015-16, haploid induction efforts expanded to ten onion varieties, with 246 plants regenerated and 90 haploids confirmed. Post colchicine treatment, 75 plants survived, and three DH lines (N-2-4-1, Agrifound Rose, and Bhima Raj) were hardened and maintained for seed production and hybridization (Table 11). These results confirm the repeatability and scalability of gynogenesis and DH production, though survival after colchicine treatment remains a limiting step (DOGR, 2015-16).

Table 11. Generation of haploids by gynogenesis and ploidy analysis of gynogenic plants

Variety	No. of flower buds cultured	No. of shoots regenerated	Number of plants tested	Haploid plants
Agrifound Rose	1152	60	34	19
Arka Kirthiman	1024	9	04	0
Arka Lalima	495	-	-	-
Bhima Kiran	2535	93	46	33
Bhima Raj	760	23	12	11
Bhima Shakti	1320	10	06	4
Bhima Shweta	1550	39	13	7

Variety	No. of flower buds cultured	No. of shoots regenerated	Number of plants tested	Haploid plants
Bhima Shubhra	520	2	-	-
MSA-3	356	1	-	-
N-2-4-1	352	9	42	16
Total	**10064**	**246**	**157**	**90**

In 2017-18, among 10,944 flower buds inoculated, 283 plantlets were regenerated and 84.29% (118/140) tested plantlets were confirmed as haploids (Table 12). Though colchicine treatment was applied for chromosome doubling, field-level success remained modest, with only one DH plant setting seed from 20 planted bulbs. In 2020, gynogenic efficiency ranged from 3.1-4.5% across five varieties, and treatment with 5'-Azacytidine (100 µM) enhanced haploid induction, especially in inbred BDR (from 4.05% to 5.54%), indicating that epigenetic modulation can improve gynogenesis. In 2021, detailed data from cultivars like Bhima Shweta, Bhima Super, and Bhima Dark Red revealed high regeneration from inbred lines compared to open-pollinated varieties, confirming genotype-dependent response (Table 13). In 2022, 67 haploids were generated from five cultivars, with Bhima Dark Red showing the highest response (24 haploids). Currently, sixty-seven doubled haploid lines have been maintained at the institute (DOGR, 2022).

Table 12. Gynogenic efficiency and haploid confirmation in 2017-18

Variety	No. of explants	Shoots Regenerated	Haploids Confirmed	% Haploids
Bhima Shweta	1768	87	35	94.59
Bhima Raj	1937	93	32	80.00
Bhima Dark Red	2192	42	16	80.00
Bhima Kiran	1795	8	7	100.00
Bhima Super	1476	15	6	66.67
Bhima Red	1431	37	21	80.77
Bhima Shakti	345	1	1	100.00
Total	**10944**	**283**	**118**	**84.29**

Table 13: Shoots regenerated in 2021 from different inbred lines and varieties

Cultivar	Inbred/Variety	Flowers Inoculated	Shoots Germinated
Bhima Shweta	I_2	1250	175
Bhima Super	I_3	1289	132
Bhima Dark Red	I_3	1181	141

Table 14. Summary of Gynogenesis and DHs development in Onion at ICAR-DOGR

Year	Varieties/Inbred Lines	Shoots Regenerated	DHs Obtained / Treated for Doubling
2005-2011	NHS, N-53, N-2-4-1	Few	Initial haploids; colchicine protocol optimized
2011-2014	Bhima Super, N-2-4-1	-	Several haploids; DHs confirmed by SSR
2014-15	5 common varieties + 1 multiplier (including Bhima Kiran)	250	9 DHs (3 field-tested)
2015-16	10 varieties (incl. N-2-4-1, Agrifound Rose, Bhima Raj)	246	3 DHs (survived and hardened)
2017-18	B. Kiran, B. Dark Red, B. Raj, B. Shweta, B. Red, B. Super, B. Shakti	283	1 DH plant set seed (out of 20 bulbs)
2020	5 varieties incl. inbred BDR, inbred BS	80 gynogenic shoots	15 DH lines maintained
2021	Bhima Shweta, Bhima Super, Bhima Dark Red (inbreds + varieties)	979 shoots (sum of selected lines)	-
2022	Bhima Shweta, Bhima Super, Bhima Dark Red, Bhima Raj, Bhima Shakti	Not specified	67 haploids (noted, not DHs)

In conclusion, the two-decade journey at ICAR-DOGR shows progressive optimization of gynogenesis-based doubled haploid production in onion. While genotypic variability, colchicine sensitivity and fertility recovery remain partial constraints, innovations like epigenetic priming and liquid rooting protocols offer strong promise. The consistent responsiveness of genotypes like Bhima Dark Red, Bhima Super and N-2-4-1 and successful field-level survival of select DHs mark a mature and scalable breeding strategy. With further molecular and physiological fine-tuning, this platform is well-positioned to significantly accelerate hybrid development in onion breeding programs (Table 14).

10. Future Strategies

ICAR-DOGR is targeting to increase the productivity up to 270% and export up to 125 lakh tons in 2050 (DOGR, 2015). F_1 hybrids will play an increasing role in future breeding because of the advantages to the breeder of exclusive ownership of the parent lines. The full exploitation of the potential of F_1 hybrids is hampered by the difficulties of commercial seed production from relatively weak parent inbreds. Further biometrical work is needed to predict the vigour of recombinant inbreds derivable from appropriate crosses; in parallel, more rapid ways of obtaining homozygous inbred lines, i.e., by anther culture, is required. There are obvious dangers in relying on a male

sterility system based on a single sterile cytoplasm type, especially one which shows some evidence of instability. Work is needed at the molecular level to determine the biochemical basis for male sterility and hence possible ways of manipulating and controlling (Gupta et al. 2017).

Male sterile lines developed at various institutes need to be tested at different locations for stability and transferred in to the background of best selected varieties and genotype with diverse nature along with development of maintainer lines. Constant up-gradation and improvement of parental lines by reciprocal recurrent selection and introgression of genes from long day types will elevate the genetic potential of parental lines. Development of 100% homozygous lines through haploidy by anther culture and diploidization will be the best option for development of quality inbred lines. Training of farmers for seed production and making available quality seed of F_1 hybrids at reasonable rates is the need of the day. Besides, potential of F_1 hybrids needs to be demonstrated through frontline demonstrations vis-à-vis farmers own material and released open pollinated varieties. This will definitely lead to successful commercialization of F_1 hybrids.

As part of the future strategies, the mode of action will focus on mobilizing support from existing funding sources to advance hybrid onion development. Firstly, funding will be sought from the ICAR corpus fund to support two specific aspects-one dedicated to infrastructure development, particularly the establishment of net houses for inbred and parental line development, and another to strengthen critical facilities. Secondly, a proposal will be developed to secure financial support from the National Agricultural Science Fund (NASF) for basic research activities, including the development of male sterile (MS) lines and double haploid lines. Additionally, the ongoing Network Project will continue to play a key role in multilocation testing of developed hybrids, ensuring their evaluation across diverse agro-climatic regions. This integrated approach is aimed at strengthening the hybrid breeding pipeline and accelerating the delivery of superior hybrids.

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Identification and development of stable male sterility lines adapted to Indian agro-climatic conditions

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Abstract

Onion productivity in India remains substantially lower than that of leading onion-producing countries, primarily due to the limited adoption of hybrid varieties. The development of F_1 hybrids in onion depends on the availability of stable male sterility systems, particularly CGMS/ CMS, which enables efficient exploitation of heterosis and economical hybrid seed production. Although well-characterized and stable male sterility systems have been extensively utilized in temperate regions, their availability, adaptation and consistent expression under Indian agro-climatic conditions remain constrained. This review synthesizes current knowledge on the male sterility systems reported in onion, with particular emphasis on S- and T-type cytoplasms, their genetic control, origin and relevance to hybrid seed production. The paper critically examines key constraints limiting hybrid onion development in India, including the scarcity of locally adapted male-sterile and maintainer lines, instability of sterility expression and the poor agronomic performance of exotic long-day lines under short-day environments. The importance of identifying, characterizing and developing stable indigenous male-sterile lines using integrated morphological, cytological and molecular approaches is highlighted as a strategic priority. Strengthening these efforts is essential to accelerate hybrid adoption, enhance productivity and improve the profitability and sustainability of onion cultivation in India.

Introduction

Onion (*Allium cepa* L.), with a chromosome number of 2n=2x=16, is a bulbous crop that belongs to the Alliaceae family and the order Asparagales. It is the second most lucrative vegetable crop globally, following tomatoes. Among *Alliums*, onions have the highest economic potential, accounting for nearly 70% of the global market share (FAOSTAT, 2022). This widespread popularity can be attributed to their rich nutritional profile, particularly their content of dietary flavonoids and quercetin compounds (Slimestad et al., 2007). Additionally, onions have several medicinal properties, including their ability to induce lacrimation, as well as anti-cancer, antibiotic and anti-cholesterol effects (Griffiths et al. 2002; Perez-Gregorio et al. 2010).

Onion is an important crop in India, playing a crucial role in export trade with a foreign exchange value of 336 million USD (APEDA, 2021). India mainly exports onions to countries like Bangladesh, Malaysia, the UAE, and Sri Lanka. It is cultivated across an area of approximately 16.2 lakh hectares, yielding around 266.4 lakh metric tons (MT), with an average productivity of 16.4 t/ha (NHB, 2021). Over the past decade, onion production has seen significant growth from 186.5 lakh MT in 2011-12 to 266.4 lakh MT in 2020-21, primarily due to an expansion in cultivation area rather than improvements in productivity, which has remained stagnant around 16 to 17 t/ha. However, this productivity level is considerably lower than that of leading onion-producing countries such as the Republic of Korea (79.6 t/ha), the Netherlands (49.7 t/ha), and other high-yielding regions like the USA, Australia, and Spain (FAOSTAT, 2022). The main reason for their higher yields is the widespread use of hybrid onion varieties.

Hybrid onions are primarily grown in temperate regions, while open-pollinated varieties are mainly cultivated in the subtropical and tropical climates of Asia and Africa (Lyngkhoi et al. 2021). In India, the hybrid onion market share remains below 1% (Khar *et al.* 2022), highlighting the urgent need to promote hybrid cultivation to enhance productivity. The development of hybrid onions largely depends on the exploitation of heterosis, which is facilitated through the use of stable male sterility systems, particularly cytoplasmic-genic male sterility (CGMS) or cytoplasmic male sterility (CMS). In temperate countries, the availability of stable male sterile systems aids in the development of hybrids. However, in countries like India, there is an urgent need to accelerate research efforts to develop and identify more stable male sterile lines with better marketable traits. This article discusses various mechanisms of male sterility in onion and emphasizes the importance of identifying and developing stable male sterility systems that are suitable for Indian conditions. It also explores the role of molecular markers in effectively developing and stabilizing these systems, which will facilitate the large-scale production of hybrid onions in India.

Different male sterile system available in onion

Different systems of male sterility have been discovered in onion. The first report of male sterility in onion was in the variety Italian Red from which the male-sterile line "13-53" was isolated, which was classified as the CMS-S type (Jones and Emsweller, 1936). Further studies described CGMS as being controlled by the interaction of male-sterile (*S*) cytoplasm with a homozygous recessive nuclear fertility-restoration (*msms*) locus (Jones and Clarke, 1943). A dominant allele (*Ms*) restores male fertility, while plants with *S msms* remain sterile. To maintain sterile lines, male-sterile plants (*S msms*) are crossed with male-fertile plants (*N msms*), referred to as maintainer lines (Jones and Davis, 1944). Since the release of the first male-sterile inbred lines in 1952, CGMS has been widely used in hybrid onion seed production (Goldman et al., 2002). The origin of *S* cytoplasm is debate suggesting it was introgressed from another *Allium* species, the triploid top-setting onion "Pran" (Havey, 1993). Alternatively, *S* and *N* cytoplasms may have coexisted naturally in wild onion progenitors (Havey and Kim, 2021). *S* cytoplasm is the most commonly used CMS type due to its stability, no reduction in female fertility, and the high occurrence of the recessive nuclear fertility-restorer allele (*msms*), which allows easy maintenance of sterile lines.

A second CMS type, T cytoplasm, was first described by Berninger (1965). Unlike *S* cytoplasm, male sterility in *T* cytoplasm is controlled by three nuclear fertility-restorer loci (Schweisguth, 1973). Although *T* cytoplasm has been used commercially, it is less common than *S* cytoplasm (Havey, 1994). De Courcel et al. (1989) proposed that *N* and *T* cytoplasms originated from a progenitor M cytoplasm, with T cytoplasm being a more recent variant. Kim and Kim, (2019) identified variants of T cytoplasm, such as "T," "T-like," and "Y," which exhibit differences in mitochondrial gene organization (*orf725* and *coxI*). These findings suggest additional, yet uncharacterized, CMS types in onion. There are other studies indicating additional sources of CMS in onion apart from genetically characterized S and T cytoplasm (Havey and Kim 2021). Another potential source of cytoplasmic-genic male sterility (CMS) originated from the 'Rijnsburger' onion, a widely grown variety in the Netherlands. This CMS type is distinct from the commonly used S cytoplasm and could offer an alternative for hybrid breeding programs (Havey, 1993). In India CMS in the open-pollinated variety Nashik White Globe was reported (Pathak and Gowda, 1993). Molecular studies showed that this CMS type is similar to S cytoplasm, possessing mitochondrial DNA fragments of the same size (Havey, 2000).

The importance of a male sterile system in the development of onion hybrids for Indian agro-climatic conditions:

Cytoplasmic-genic male sterility (CGMS) is a crucial system for hybrid onion seed production, as it eliminates the need for manual emasculation, which is impractical due to the small size of onion flowers and the extended blossom period. The onion umbel consists of hundreds of small perfect flowers that shed pollen when adjacent flowers have receptive stigmas, making large-scale emasculation highly laborious. Apart from this the development of hybrid onions is limited due to the biennial reproductive nature of the crop, involving a seed-to-bulb followed by a bulb-to-seed cycle. The high rate of out crossing, the difficulty in maintaining purebred lines and the occurrence of inbreeding depression upon repeated selfing. Also on emasculation, seed output per cross is limited to a maximum of six seeds per flower. Additionally, the extended blossom period of more than two weeks contributes to open-pollination, reducing hybrid purity.

The phenomenon hybrid vigor in onions has been successfully exploited in countries such as the USA, Japan, Korea and the Netherlands this is due to availability of long day male sterile lines. Exotic long-day male-sterile lines perform poorly under short-day conditions in India. Additionally, the lack of locally adapted male-sterile lines with maintainer genotypes has further hindered the hybrid breeding program at the national level. It is also reported that, the limited availability of maintainer lines, instability of male sterility, labor-intensive seed production and complex genetic factors have restricted hybrid adoption in other regions of the world except temperate countries (Manjunathagowda et al. 2021). Hybrid onions are predominantly cultivated in temperate regions, while open-pollinated varieties continue to dominate subtropical and tropical areas such as Asia and Africa. In India, the market share of hybrid onions remains below 1% (Khar *et al.,* 2022), highlighting the need for developing stable male-sterile lines adapted to Indian agro-climatic conditions to improve hybrid onion adoption and productivity.

The occurrence of the N*msms* genotype in local populations is comparatively rare (van der Meer,

1993; Manjunathagowda et al. 2021). Identifying male-sterile lines based on phenotypic traits is relatively easy, but extracting maintainer lines from an onion population is a major challenge due to the biennial nature of onion, high frequency of the dominant allele at the *Ms* locus, and the prevalence of S cytoplasm (Satoh et al. 1993; Havey and Randle, 1996). To enhance hybrid onion production in India, focused research is required to identify and develop stable male-sterile lines and their respective maintainer and restorer line suitable for Indian agro-climatic conditions. This will not only facilitate hybrid seed production but also contribute to increasing onion productivity and profitability for Indian farmers.

Morphological and cytological differences between male sterile and fertile plants

There are certain morphological and cytological features that can be either observed through naked eyes or using microscope, these features that are distinct between male fertile and male sterile onion plants are listed in following table.

Trait	Male Fertile Plant	Male Sterile Plant	Reference
Plant Vigour	More vigorous	Less vigorous	Manjunathagowda and Anjappa, 2020
Bolt Height	Taller	Comparatively shorter	
Umbel Diameter & Height	Larger and higher	Smaller and lower	
Flower Arrangement	Wider spread from the central head of umbel	Compactly arranged with central head	
Petiole Length	Longer	Shorter	
Perianth Segment	Fully open and enlarged	Slightly curved and less open	
Style Length	Medium, at the height of filaments	Longer than filaments	
Stigmatic Surface	Wider	Narrow stigmatic knob	
Anther colour and surface	Prominent, dark green or yellow colour with rough surface	Light green colour, brown and shrunken anthers with smooth surface	Ahmed et al. 2020
Pollen Viability	Viable and functional and viable pollen stain red using acetocarmin staining	Non-viable on acetocarmine test they stain nil or slightly, pollen degeneration starts at the tetrad stage	Kim, 2014; Saini et al. 2015
Microspore Development	Normal	Degeneration at tetrad stage, pollen sterility	Pathak, 1997; Patil et al. 1993
Tapetal Cells	Normal	Hypertrophied in male-sterile variety Niphad 2–4–1	Patil et al. 1993
Special Features	No bulbils in umbel	Sterile flowers with top-set bulbils	Jones and Emsweller, 1936

Molecular markers available to distinguish male sterile and fertile lines

Onions possess cytoplasmic-genic male sterility (CGMS), which is widely used in hybrid seed production. The ability to distinguish male-sterile and fertile lines at the molecular level is crucial for efficient breeding programs. Several molecular markers have been developed for the identification of S-, T- and N-cytoplasm in onion, enabling breeders to accurately classify plant lines without relying solely on morphological traits. Similarly, identification of molecular markers closely associated with the fertility restoration *Ms* locus has greatly facilitated the breeding of onion maintainer lines (Havey, 2013). Employment of PCR markers for the determination of cytoplasm and nuclear *Ms* locus is highly recommended for accelerated hybrid development (Khar et al. 2022).

The markers that differentiate N-cytoplasm from sterile S-cytoplasm a cytoplasmic identifier derived from the *cob* mitochondrial gene and a chimera gene, which included an insertion of chloroplast DNA into the upstream region of cob in the S-cytoplasm (Sato, 1998) is most used marker to differentiate N- and S-cytoplasm. Nonetheless, the 5' *cob*-marker fails to differentiate T-cytoplasm from N-cytoplasm. There are also markers that are facilitating identification of three cytoplasmic types based on DNA sequences of additional mitochondrial genes, including the cox1 gene, which encodes cytochrome c oxidase subunit I, and its chimeric or f725 gene (Kim et al. 2009). The *Ms* locus and its segregating types can be selected using RFLP, SCAR and PCR markers like SNPs and SSRs (Gokce and Havey 2002; Yang et al. 2013; Bang et al. 2011, Khar et al. 2022). The list of PCR markers available to distinguish male sterile and fertile lines in respective to cytoplasm and *Ms* locus have been listed in following table.

S. No	Primer	(F and R)	Sequence	Identifier	Band size	References
1	OPT	F	CCTTGGAAAGGCGCAA CTAAAGATTTGA	*Ms* Locus	MsMs-659bp msms-526bp	Bang et al. 2011; Khar et al. 2022
		R	TGTGGCCCAATAATACA AACAAGCAGGA			
2	OSN/Cob	Normal (N) F	TCTAGATGTCGCATCAG TGGAATCC	Cytoplasm	N-180bp S-414 bp	Sato, 1998, Saini et al. 2015; Khar et al., 2022
		Sterile (S) F	GTCCAGTTCCTATAGAA CCTATCACT			
		Common R	CTTTTCTATGGTGACAA CTCCTCTT			
3	MK	F	CAT AGG CGG GCT CAC AGG AATA	Cytoplasm	N-833 bp, S-628 bp	Kim et al. 2009
		R1	AAT CCT AGT GTC CGG GGT TTCT			
		R2	CAG CGA ACT TTC ATT CTT TCGC			

S. No	Primer	(F and R)	Sequence	Identifier	Band size	References
4	AcPMS1	F	GGT CAC CAG GTG GAT AGA GAA	*Ms* Locus	MsMs-242 bp, msms-276 bp	Kim et al. 2015; Khar et al. 2022
		R	TCA TTG AGC TGC ATC CAA AA			
5	atp9	F	CGGCTGTCGGTATTGG AAACG	Cytoplasm	-	Engelke and tatlioglu et al. 2002
		R	GATCAGAAAGGCCATCA TTAGG			
6	*orf*A501 gene	F	ATGGCTCGCCTTGAAAG AGAGC	Cytoplasm	-	Engelke et al. 2003
		R	CCAAGCATTTGGCGCTG AC			
7	PsaO gene	F	CCTCATGCTTGCTTGGT CTT	*Ms* Locus	-	Bang et al. 2011
		R	AAGCGTGATCGATTGTA GGTCCTTT			
8	Rf gene	F	TCACCTTTTACTTGCATC TGGTT	*Ms* locus	-	Kim, 2014
		R	CCATTGGTACTTGATGC AAA			

Ways and means in identification and development of stable male sterility lines

The identification of male-sterile and maintainer lines in diverse genetic backgrounds serves as the foundation for onion hybrid development programs. The isolation and maintenance of male-sterile and maintainer lines from open-pollinated varieties or landraces offer an effective approach for onion breeders to develop hybrids. The occurrence of male sterility in open-pollinated genotypes may result from *ms* gene migration through natural cross-pollination. In conventional method of identification and development of male sterile line, male-sterile trait in onion being cytoplasmically inherited, while fertility restoration depends on nuclear genes. Plants with normal fertile (N-type) cytoplasm are always fertile, regardless of whether the fertility gene (*Ms*) is dominant or recessive. Male-sterile lines (S*msms*) can only be maintained by crossing them with maintainer plants possessing N-type cytoplasm and homozygous recessive genes (N*msms*) at the *Ms* locus (Havey, 2013; Jones and Davis, 1944). Identifying better maintainer lines is critical for the successful maintenance of male-sterile lines and hybrid seed production. For identification, male-sterile lines (S*msms*) are crossed with male-fertile (N- or S-*Ms*) plants. If all test cross progenies are fertile, the male parent is homozygous dominant for

the *Ms* locus. Conversely, if the progenies are sterile, the male parent is N*msms* and can serve as a maintainer line (Havey, 2013). Maintainer lines are genetically identical to male-sterile plants, except for their normal fertile cytoplasm. Once these sterile lines and respective maintainer lines are identified these lines are selfed for few generations to attain homozygosity. Care should be taken not to lose the vigour of identified plant while selfing as onion has high inbreeding depression.

The development and evaluation of test cross progenies for the homozygosity of the recessive *Ms* locus in N cytoplasm (maintainers) typically take 4-8 years, given that onion requires two growing seasons to complete one cycle. This process is time-consuming and inefficient compared to modern molecular techniques. The availability of tightly linked molecular markers and marker-assisted selection (MAS) has significantly accelerated onion improvement programs. Pollen viability tests and molecular markers can be effectively used to identify these male-sterile lines and their corresponding fertility restorer lines. Molecular markers distinguishing normal (N) versus sterile (S) have been identified in onion. Identifying molecular markers tightly linked to the nuclear Ms locus has greatly facilitated the development of maintainer lines. For example, in ICAR-VPKAS Almora, PCR based markers, 5' cob-based marker amplified 180- bp fragment in N cytoplasm whereas in S cytoplasm amplification of two fragments (180 and 414bp) was used. Another PCR based marker of OPT, linked to the restorer of fertility (Ms) locus (Bang et al. 2011) was used to distinguish fertility restorer (Ms) locus. Fertility/sterility of plants were validated by acetocarmine staining of the pollens and correlated with observations made using molecular markers. Only 7 plants happened to be completely male sterile and single maintainer plant was identified in VL Piaz 3. This is the first example of deploying DNA markers for identification and purification of male sterility and hybrid development in long/ intermediate day length onion in Indian population. Selected sterile line designated as VL In. 31-1A (CMS Female) & VL In. 31-1B (Maintainer) (IC645760 & IC645761; INGR22084) and registered as genetic stock with NBPGR (Hedau et al. 2024). These markers enable breeders to detect plants carrying recessive *ms* alleles in the first year itself with N and S cytoplasm that can be used as sterile and maintainer lines in three-way hybrid production.

Conclusion

Development and identification of stable male-sterile lines in diverse background (Short day/ intermediate/long day type) adapted to Indian agro-climatic conditions is essential for advancing hybrid onion cultivation. To overcome this, breeding locally adapted male-sterile and maintainer lines is crucial. Molecular markers can further enhance hybrid breeding by enabling precise differentiation of male-sterile and fertile lines. Focused research on identifying suitable male sterile lines, combined with molecular tools, will support large-scale hybrid seed production, ultimately improving onion productivity and strengthening India's global market position.

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Challenges in development of suitable maintainer lines for onion hybrid breeding

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Abstract

The success of F_1 hybrid breeding in onion is critically dependent on the availability of stable cytoplasmic male sterile (A) lines and their corresponding maintainer (B) lines; however, the identification and development of suitable maintainers remain a major breeding bottleneck. Onion is a highly cross-pollinated and heterozygous species with a large genome size, which results in a low natural frequency of maintainer genotypes and pronounced inbreeding depression during line development. The biennial growth habit further extends breeding cycles, while the lack of reliable and universally applicable morphological or molecular markers complicates early and accurate identification of maintainer plants. Although several DNA markers linked to cytoplasmic male sterility and fertility restoration have been reported, their utility is often population-specific. In India, predominance of N-cytoplasm offers a relative advantage, yet weak linkage of available markers necessitates development of indigenous, robust marker systems. Integration of association mapping, speed breeding and doubled haploid production through gynogenesis holds promise to overcome these challenges. Strengthening these approaches will be crucial for accelerating maintainer line development and expanding onion hybrid breeding in India.

Introduction

Onion hybrid breeding has revolutionized onion cultivation in many parts of the world. Owing to F_1 hybrids, countries like the US and Japan have been deploying F_1 hybrids to increase their yields, productivity and quality since long. The global onion seed market is continually evolving with a tendency towards hybrids and hybrid derivatives. In 2022, more than 85 per cent of onion seed market was shared by hybrids. Where, in the US and Canada, the adoption rate of onion hybrids is about 98%, countries like India and China despite being the leading onion producers are witnessing much lower hybrid adoption rates of 57 and 45 per cent, respectively. In Europe and many other countries, open pollinated (OP) varieties are still dominating the F_1 hybrids. There are many reasons for different adoption rates in different parts of the world. One of them is trend towards organic farming which requires less nutrient consuming varieties whereas, F_1 hybrids require higher fertilizer

inputs. Better adaptability of OP varieties to local agro-climate also makes them the first choice among growers. Another major reason is larger proportion of small and marginal farmers in many countries who cannot afford costly F_1 seeds. Despite varied outlook of hybrid adoption across the globe, F_1 hybrids are able to perform better than OP varieties in terms of being more productive, uniform and resistant in general. There are reports of heterosis for yield, quality and disease resistance in onion hybrids over OP varieties.

Many Indian onion growers are now preferring F_1 hybrids over OP varieties and the demand is expected to rise. To meet the supply targets in near future, research on hybrid development needs to be strengthened in India. It goes without saying that core requirements for onion F_1 hybrid development are A and B lines. The A or male sterile line acts as female parent of the hybrid while B or maintainer line propagates the A line without disturbing its nuclear male sterile genotype (*msms*). In the absence of maintainer line (N*msms*), nuclear fertility restorer/ sterility alleles of male sterile line (S*msms*) may segregate to yield at least 50 per cent male fertile progeny disrupting the maintenance of male sterility of A line. A line or the female parent of the hybrid is identical to B-line at the genetic level with the only exception of sterile cytoplasm with B-line having male fertile cytoplasm. Therefore, the importance of maintainer line cannot be overemphasized. Developing a maintainer line is as important as developing a male sterile line. However, as much as maintainer line is crucial for hybrid development, it is equally challenging to develop it in onion. The major challenges faced by onion breeders in developing maintainer lines have been discussed under the following headings.

High level of cross pollination

Onion is well-known for its highly out crossing nature. Protandry and honeybee activity ensures that the flowers experience more than 90 per cent cross pollination (Oshone and Kalsa 2023). This high level of cross-pollination necessitates arrangements to prevent foreign pollen while working with plants meant for male sterile and maintainer line development. Cross-pollination also leads to heterozygosity and heterogeneity of the species, which further complicate the breeding of such crops.

Highly heterozygous and large genome

High degree of cross-pollination has led to high level of heterogeneity as well as heterozygosity in onion genome. Heterozygosity of the genome presents difficulties in obtaining true breeding lines whether for use as a variety or a maintainer line. There is more chance of the trait of interest being heterozygous than homozygous. That is why, frequencies of male sterile or maintainer homozygotes (S*msms* and N*msms*, respectively) are found to be much lower than the heterozygotes (*MSMS*) in all onion populations across the world. The very low frequency of maintainer genotype makes its discovery from a large population a rare event in itself. The very large genome of onion equaling 16 giga base pairs is a very significant challenge compromising the development of genomic tools for onion breeding including marker assisted selection for parental lines.

Experiences strong inbreeding depression

Highly cross-pollinated species like onion and carrot have attained vigour and resilience to tough environments by achieving heterozygosity for most of their loci *via* out-crossing. Repeated forced selfing in such crops tends to reveal lethal expressions of recessive alleles by promoting homozygosity. This disrupts the natural balance of species' fitness and it undergoes inbreeding depression more abruptly and intensely than the predominantly self-pollinating species. In onion, a maintainer line should have all the economic traits desired by the breeder along with a good combining-ability because eventually all these traits are going to be there in the female parent of the hybrid. It is therefore evident that development of a competent maintainer line is a challenging task in the face of inbreeding depression. Higher the level of desired homozygosity, more the number of selfings required, which works against the aim for a vigorous B line.

Lack of markers linked to maintainer genotype

There are no morphological markers linked to maintainer (N*msms*) genotype that could help identify maintainer plants in nursery or field. To identify them, a breeder must rely on test crossing random plants with an established male sterile plant/ line. The results of these test-crosses become evident after 2-4 years. This method is not only time-taking and cost-ineffective, it may not yield desired results at the end of such long waiting period, as the plants selected for test-crosses are not based on any criterion. Considering this major bottleneck, some molecular markers were developed, which could assist in identifying maintainer genotype at seedling stage itself. RAPD marker jnurf20, CAPS markers jnurf05 and jnurf17, locus specific markers like PsaO, OPT, ACms 1100, DNF-566, RNS-357, high resolution markers and high through-put multiplex markers gradually came into existence (Park et al 2013; Bang et al 2011; Bang et al 2013; Yang et al 2013; Kim et al. 2019). However, there are mixed reports of their applicability to different populations across the world. Also, these are very few in number and need to be developed for each genetic background for utility in most populations of the world. The simultaneous detection of sterile cytoplasm and corresponding fertility restorer locus is, however, of immense value in onion F_1 hybrid breeding and development of HRM and multiplex markers have paved path for the same.

Biennial life cycle

Onion is inherently a biennial crop if grown for seed. For identifying maintainer plants and developing them into a line, whole biennial life cycle from seed to seed is to be tapped. So, the breeder has to put in efforts and time for 2 years to confirm the nuclear status of maintainer plants he/ she had selected at the start of the program. To address this difficulty, several molecular markers based on cytoplasmic and nuclear DNA have been developed. However, as discussed earlier, all the markers are not effective for all populations of onion across the globe. So, there is a need to develop molecular markers for onion germplasm in every part of the world to establish robust markers to detect and isolate desired fertility restorer allelic status (*MSMS/ msms*).

Conclusion

Although there are many difficulties in isolating maintainer lines in onion, India is still in an easy position because studies on Indian onion populations using cytoplasmic markers have shown predominance of N cytoplasm over S cytoplasm (Sheemar and Dhatt, 2015 and Khar et al. 2022). There is only one report (Khar et al. 2022) of T cytoplasm in India from T821, a private F_1 hybrid, which probably traces its origin out of India and S-cytoplasm is always in much smaller proportion. India may now focus more on establishing its own DNA based markers since linkage disequilibrium between markers reported from elsewhere in the world and *MS/ms* locus in Indian onions seems to be weak (Khar and Soni 2016; Malik et al. 2023). Intensification of association mapping studies in onion will assist in achieving this target.

Speed breeding can be another useful approach to cut short the breeding cycle of onion. Varying climatic conditions like mean temperatures and day lengths are available in India to make use and may be wisely used to speed up the development of maintainer lines for onion F_1 breeding.

Development of doubled haploids (DH) is a very useful strategy to overcome inbreeding depression in onion and it can very well be used for developing maintainer lines. Gynogenesis circumvents repeated selfing thereby avoiding successive inbreeding depression that the inbred line has to undergo in each generation. Almost 100 per cent homozygosity can be achieved *via* gynogenesis and the development of a maintainer or any inbred line becomes effective and efficient. Gynogenesis, however, has been reported to yield varied results depending on genotype, climate and media composition (Dhatt and Thakur, 2014). A study on Indian short day tropical onions was done by Khar et al. 2018, which showed good response of some genotypes to callus formation, embryo induction, shoot multiplication and pot soil hardening. India has different agroecological zones and length responsive onions. A collaborative approach to developing doubled haploids in Indian onion can be taken up, as it will have higher chances of achieving DH from one or the other genetic background.

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Molecular tagging and marker-assisted selection for introgression of male sterility genes in Indian onion genotypes

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Abstract

Onion is a globally important vegetable crop, yet its genetic improvement is inherently constrained by a biennial growth habit and severe inbreeding depression. The identification and exploitation of CMS systems have provided an effective and economic framework for large-scale hybrid seed production in onion. Several CMS cytoplasms, namely S, T, R and N have been reported, of which the S-type cytoplasm is most extensively utilized in India due to its stable expression and simple fertility restoration controlled by a single dominant *Ms* gene. However, conventional development of male sterile (A) and maintainer (B) lines is laborious and typically requires 10-12 years. Marker-assisted selection (MAS) offers a robust alternative by enabling precise and early identification of cytoplasmic types and nuclear fertility restoration alleles, thereby substantially shortening breeding cycles. Several PCR-based molecular markers, including 5' *cob*, *orfA501*, *orf725*, *jnurf13* and *AcPMS1* have been developed and validated for reliable discrimination of CMS sources and fertility restoration alleles. The deployment of these markers has markedly accelerated the introgression and maintenance of male sterility systems in onion breeding programs. Notably, at PAU, Ludhiana, MAS-based selection facilitated the rapid isolation of CMS, maintainer and restorer lines from locally adapted OP populations, leading to the development of the high-yielding hybrid Punjab Onion Hybrid-1 (POH-1). These outcomes underscore the effectiveness of molecular tagging and MAS in improving the efficiency, precision and sustainability of hybrid onion breeding in India.

Introduction

Onion (*Allium cepa* L., 2n=16) is the third most important commercial vegetable globally, valued for its medicinal benefits, including blood sugar regulation, platelet aggregation inhibition, and antioxidants like quercetins and flavonoids. Despite its significance, onion genetics and genomics lag due to its biennial cycle and severe inbreeding depression, which complicate breeding. In India, breeders extensively utilized mass selection to breed open-pollinated varieties, whereas F_1 hybrids are prevalent in the USA. The discovery of cytoplasmic male sterility was made by Henry Albert Jones and paved the way for cost-effective commercial hybrid onion seed production (Jones and

Emsweller, 1936). In onion for F_1 seed production different types of cytoplasm, *i.e*. CMS-S, CMS-T, CMS-N, and CMS-R types are utilized (Havey and Kim, 2021). The CMS-S type cytoplasm was first identified in the onion cultivar 'Italian Red', found in its pedigree '13-53' (Jones and Emsweller 1936). The CMS-S cytoplasm has a simple inheritance of male fertility restoration and is governed by a single dominant (*Ms*) gene at the nuclear restorer locus (Jones and Clarke, 1943). The S-cytoplasmic plants have the male-sterile cytoplasm and are homozygous recessive (S*msms*) at the restorer locus (Jones and Clarke, 1943). It has been proposed that S-cytoplasm is transferred into onion from another *Allium* species "Pran" that is found in Indian Kashmir region (Jones and Emsweller, 1936; Havey, 1993).

The CMS-T type was reported by Berninger (1965) in a French cultivar 'Jaune paille des Vertus'. In the CMS-T cytoplasm, the inheritance of restoration of male fertility (MF) is complex, and controlled by two complementary genes (*B/b-C/c*-) and one independent (*A/a*-) gene (Berninger, 1965; Schweisguth, 1973). Recently, 'T-like' cytoplasm was labelled as 'CMS-R' cytoplasm due to that having originated from the population of onion cultivar 'Rijnsburger' in the Netherlands, and it seems to be more identical to the T- and N- cytoplasm than the S- cytoplasm (Havey and Kim, 2021). The S-type is the most commonly used cytoplasm worldwide, including India, for hybrid onion cultivar development, whereas the R-type is exploited in European countries, and the T-type cytoplasm is rarely used (Havey and Kim, 2021). Cytoplasm from *A. galanthum* has been introduced into onion and this offers an alternative source of male sterility for onion breeding, broadening the diversity of available CMS systems (Havey 1993). In *A. galanthum* cytoplasm male sterile lines lack anthers, simplifying the identification of sterility in comparison to the existing S- and T-type cytotypes.

Due to the biennial life cycle of onions, developing male sterile (A-) and maintainer (B-) lines through conventional breeding methods can take 10-12 years (Santos et al. 2010), making the process labour-intensive, tedious, and time-consuming (Figure 1). The marker-assisted selection allows cytotytpe identification and isolation of maintainer (N*msms*) line from the same population at the seedling stage (Havey 1995, Kim et al. 2009). Researchers have developed and identified diverse molecular markers (Table 1) to distinguish different sources of cytoplasm(s) *viz.* N-, S-, R- and T- and also genotypes at *Ms* locus (de Courcel et al. 1989; Kim *et al* 2015; Sato 1998; Von Kohn *et al* 2013). The prominent marker cp *accD* (Von Kohn et al. 2013) was recommended to separate the S- cytoplasm from other onion cytoplasm(s). A PCR-based molecular marker 5' *cob* developed by Sato (1998) easily distinguishes the male sterile S- and N- cytoplasm. Later, Engelke *et al* (2003) developed a molecular marker i.e. *orfA501*, which is capable of quick and confident identification of the S-, T-, and N- cytoplasm of individual plant(s) of an open-pollinated population. The CMS R- cytoplasm is distinguished from the other cytoplasm(s) by using *orf*725 marker (Kim *et al* 2009). Two indel markers, namely, *jnurf13* (Kim, 2014) and *AcPMS1* (Kim et al. 2015) were reported to co-segregate with *Ms*. The reported tightly linked molecular markers could be utilized in marker-assisted selection (MAS) of cytoplasm types and *Ms* locus. Punjab Agricultural University (PAU), Ludhiana, successfully isolated CMS (A-), maintainer (B-), and restorer (R-) lines from locally adapted open-pollinated onion populations using marker-assisted selection (Sheemar and Dhatt 2015). Using the CMS lines, PAU has developed a high yielding onion hybrid POH-1 (Punjab Onion Hybrid-1).

Table 1. Molecular markers linked to different cytotypes and *Ms* locus in onion

Cytotype and *Ms* locus identification	**Type of marker**	**Reference**
N and S	RFLPs	Holford *et al*. (1988), Havey (1993), Sato *et al* (1993)
	SCARs	Havey (1995)
	PCR	Sato (1998), Lilly and Havey (2001)
	SNPs	Von *et al*. (2013)
N, S and T	PCR	Kim *et al*. (2009), Kim and Yoon (2010)
	SNPs	Kim and Kim (2019)
N, S and R	PCR	Kim *et al*. (2009); Havey and Kim (2021)
N and T	PCR	Ahn and Kim (2023)
N, S, R and T	HRM	Khrustaleva *et al*. (2023)
Ms locus	RFLPs	Gokçe *et al*. (2002)
	SNPs, InDels, SSRs, RFLP	Martin *et al*. (2005)
	SCARs	Yang *et al*. (2013)
	CAPS	Park *et al*. (2013); Bang *et al* (2013)
	SNPs	Havey (2013), Duangjit *et al* (2013)
	PCR	Kim *et al*. (2015)
	InDels	Kim (2014)
	Multiplex	Huo *et al*. (2015); Liu *et al*. (2019)
	Tyramide FISH	Khrustaleva *et al*. (2016)
Ms-2	SNP	Yu and Kim (2021)

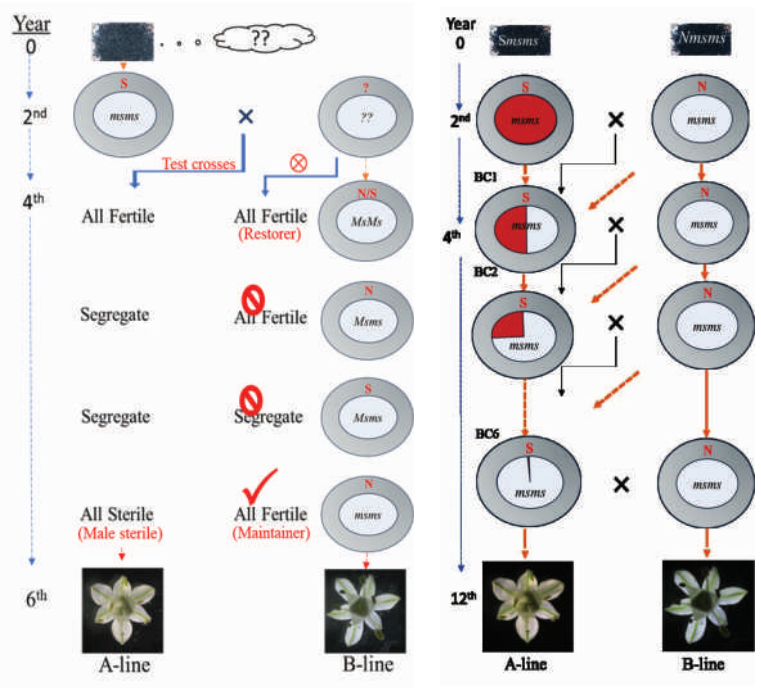

Figure 1. Conventional approaches for developing male sterile and maintainer lines. a) Development of male sterile line from open pollinated population and b) Transfer of male sterility from known source into the desired genetic background

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Application of biotechnological tools in accelerating inbred line development in onion (*Allium cepa* L.)

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Abstract

The development of homozygous inbred lines remains a critical bottleneck in onion breeding owing to its strict cross-pollinated nature, pronounced inbreeding depression and biennial life cycle, all of which substantially prolong genetic improvement programs. Conventional selfing requires six to seven generations to achieve acceptable levels of homozygosity and often necessitates intermittent population bulking to maintain plant vigour, thereby limiting breeding efficiency. The application of advanced biotechnological tools offers transformative opportunities to overcome these constraints and accelerate inbred line development. Haploid and doubled haploid (DH) production via *in vitro* gynogenesis provides a direct pathway to rapid homozygosity, although its practical deployment is currently constrained by low induction efficiency and poor seed set. Recent progress in *in vivo* haploid induction through genome elimination, particularly CENH3 based systems, represents a promising, scalable and field-amenable alternative. Furthermore, the integration of haploid induction with genome editing (HI-Edit) enables precise, transgene-free introgression of target traits, facilitating rapid genetic enhancement of elite inbred lines. Emerging evidence also implicates epigenetic mechanisms in onion inbreeding depression, with preliminary studies indicating that modulation of DNA methylation can partially restore vigour in advanced inbred generations. Collectively, the strategic integration of haploid induction technologies, genome editing, molecular tools and epigenetic interventions has the potential to substantially shorten breeding cycles, improve inbred line quality and enhance the overall efficiency and sustainability of hybrid onion breeding programs.

Introduction

Inbred lines are developed through selfing or crossing between closely related individuals over multiple generations to achieve genetic uniformity. They play a crucial role in breeding programs, serving as the foundation for developing hybrids with greater vigour and uniformity. One of the major challenges in inbred development is the time required to achieve homozygosity, which typically takes six to seven generations of selfing. This process becomes even more complex in

onion, a cross-pollinated crop that suffers from severe inbreeding depression. To prevent the loss of inbred lines due to reduced vigour, onion breeding often requires intermittent cycles of massing between selfing generations. Additionally, the biennial growth habit of onion further prolongs the time needed to develop stable inbred lines. Under such constraints, biotechnological tools can significantly enhance efficiency, accuracy, and speed in inbred development. The tools *viz*.; tissue culture, molecular markers, genomic selection, genotyping by sequencing, NGS, and genome editing can help to accelerate the process of achieving homozygosity, improve vigour and facilitate the transfer of desirable traits, complementing conventional breeding methods.

Haploid induction in onion:

In vitro haploid induction

Haploids are sporophytes that carry the gametic chromosome number of a species and commonly occur in nature. They can be artificially induced from gametic cells through tissue culture, providing a means to achieve homozygosity in a single generation.

In onion, haploid induction has been reported through *in vitro* gynogenesis using isolated flower bud culture. Additionally, *in situ* induction of maternal haploids has been achieved through pollination with irradiated pollen (Dore and Marie, 1993) and sterile pollen from triploid donors, as observed in shallot onions (multiplier type) (Endang-Sulistyaningsih et al., 1997). However, efforts to induce regeneration through androgenesis have been unsuccessful (Keller, 1990).

Among the available methods for haploid induction, isolated flower bud culture remains the most frequently used approach in onion (Campion et al., 1992; Bohanec and Jakše, 1999). This is likely due to the low efficiency of other methods compared to *in vitro* gynogenesis. Haploid induction in Indian onion genotypes has been successfully achieved in more than 15 genotypes (Anandhan et al., 2014), though the induction rate generally remains low, often below 5%. While chromosome doubling using colchicine and APM has been achieved, the poor seed set in doubled haploid (DH) lines remains a significant challenge, limiting the large-scale multiplication of haploids.

In vivo haploid induction:

Genome elimination following hybridization in interspecific crosses has been widely utilized for haploid production across various species. In maize, intraspecific haploid inducer lines have long been employed for dihaploid generation. The maize haploid inducer line has been well-characterized, and such lines can be engineered through targeted knockout of the ZmMTL gene (Kelliher *et al*., 2017). Similarly, homolog of the DMP gene from maize has been tested in tomato to develop haploid inducer lines (Zhong et al., 2021). A significant advancement in haploid induction was made with the discovery of CENH3-based genome elimination in *Arabidopsis thaliana* (Ravi and Chan, 2010), which opened up the possibility of inducing haploids in any species through targeted genome elimination. In this system, modified CENH3 lines (e.g. GFP-tailswap CENH3) act as haploid inducers by eliminating parental chromosomes with modified CENH3. This approach has recently been extended to develop

haploid inducer lines in maize. Additionally, certain CENH3 missense mutants in *A. thaliana* have also demonstrated haploid induction capability. CENH3 based haploid inducer line has been developed through RNAi based suppression of AcCENH3 (Manape et al., 2024)

These strategies-namely, genome elimination-based haploid induction and engineered haploid inducer lines in maize-offer several key advantages:

- Simplification and scalability of haploid induction in the field, reducing reliance on high-tech laboratory infrastructure and specialized personnel.
- Enhanced competition in onion breeding, potentially leading to superior varieties or hybrids with desirable traits.
- Streamlined trait transfer across inbred lines via integrated genome editing and haploid induction techniques (e.g., HI-Edit), including applications such as male sterility transfer.

Genome editing for improvement of inbreds:

Genome editing offers a powerful tool for improving inbred lines by enabling precise modification of target genes. In crop plants, genome editing typically involves the generation of stable transformants, followed by segregation to eliminate the transgene. However, recent advancements have enabled transgene-free genome editing through transient delivery methods such as biolistic transformation of ribonucleoprotein (RNP) complexes (Park and Choe, 2019), the use of nanomaterials as carriers (Chen et al., 2019), or Agrobacterium-mediated transformation (Veillet et al., 2019). While these methods are relatively simple to implement, they often require labour-intensive screening to identify successfully edited individuals.

An alternative strategy involves the development of a genome editing stock line in a transformation-proficient genotype. This stock can then be used for trait transfer when combined with a haploid inducer line. When paired with CENH3-based haploid induction (or any other haploid induce system), such an editing line can serve a dual purpose-inducing both haploidy and genome editing (Kelliher et al., 2019). The editing construct, delivered through male or female gametes, can introduce the desired genomic change in the zygote, which is then retained in the edited genome while the construct itself is eliminated through genome elimination.

This integrated approach, known as HI-Edit, holds great potential for gene function studies as well as one-step trait transfer across genotypes or even species. Ongoing research on this innovative system is being carried out in onion at ICAR-DOGR, with support from the Department of Biotechnology, New Delhi.

Epigenetic Modification to Reduce Inbreeding Depression in Onion

Onion exhibits severe inbreeding depression upon selfing, leading to poor vigour and loss of population stability (Havey, 2018). As a result, the crop typically requires massing cycles after a few

generations of selfing, significantly extending the time needed to develop inbred lines through conventional methods. Inbreeding depression is controlled by both genetic and epigenetic factors. Interestingly, in maize, signs of inbreeding depression have been observed even after many generations of selfing, when maximal homozygosity is expected-suggesting a potential role for epigenetic regulation (Han et al., 2021).

In this context, we have treated inbred onion populations with azacytidine, a known DNA demethylating agent, at various concentrations. The treatment resulted in a partial recovery of vigour in inbred lines, indicating the involvement of epigenetic mechanisms in inbreeding depression. These preliminary findings suggest that epigenetic modification could offer a novel approach for mitigating inbreeding depression and accelerating the development of viable onion inbreds.

Further research in this area holds promise for overcoming one of the major bottlenecks in onion breeding.

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Innovative and cost-effective methods of inbreds and hybrid seed production in onion

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Abstract

Onion (*Allium cepa* var. *cepa* L.) is one of the most economically significant vegetable crops worldwide, yet its productivity in India remains well below the global average due to reliance on open-pollinated varieties (OPVs), limited hybrid adoption, and systemic breeding challenges. This paper explores innovative and cost-effective approaches for inbred and hybrid seed production in onion, highlighting the potential of heterosis breeding and cytoplasmic male sterility (CMS) systems. The successful development of onion hybrids requires genetically diverse, high-performing inbred parental lines with superior horticultural traits, genetic purity, and adaptability. However, breeding true inbred lines in onion is hampered by severe inbreeding depression, biennial growth cycles, and high genetic load. These biological constraints significantly slow the hybrid development pipeline. To address these challenges, the integration of modern breeding tools such as doubled haploid technology, marker-assisted selection (MAS), and speed breeding is emphasized. A detailed overview of the onion hybrid breeding framework is presented, outlining the sequential development and evaluation of inbred lines and hybrid combinations. Adoption of these cost-effective and innovative methods is crucial to enhance hybrid seed production, improve productivity, and make Indian onion cultivation globally competitive.

Introduction

Onion (*Allium cepa* var. *cepa* L.) is one among the most commercially important horticultural crops, cultivated and consumed globally. Often referred to as the "Queen of the Kitchen" (Selvaraj, 1976), onions serve as both a staple vegetable and a spice, forming a vital part of culinary traditions worldwide. Their widespread use is attributed to their flavour-enhancing properties, nutritional value, and multiple health benefits, including antihypertensive, antidiabetic, antimicrobial, immune-boosting, and anti-obesity effects (Zhao et al., 2021). Nutritionally, onions are rich in essential minerals such as magnesium, calcium, potassium, and phosphorus, along with folic acid,

vitamin B6 (Raghu, 2023), and dietary fibre (Pareek et al., 2017). Beyond fresh consumption, onions are extensively used in the food processing industry, contributing to value-added products like dehydrated flakes, powders, and pastes (Gnanasundari et al., 2023), underscoring their economic importance.

Globally, onion ranks as the third most significant vegetable after potatoes and tomatoes, cultivated in around 140 countries over 5.48 million hectares, yielding 104.55 million metric tonnes (FAOSTAT, 2020-21). The top five producers India (26.73 m MT), China (23.72 m MT), the United States, Egypt, and Turkey account for over 50% of global production. India leads in cultivation area (1.43 million hectares), but its average productivity (18.64 t/ha) remains well below the global average (23.06 t/ha), ranking 83rd worldwide. In contrast, countries like the Republic of Korea (79.62 t/ha), the United States (71.10 t/ha), and Australia (54.67 t/ha) achieve much higher yields (FAOSTAT, 2020–21), highlighting the productivity gap in Indian onion cultivation.

This low productivity in India stems from multiple challenges: suboptimal agronomic practices, inadequate irrigation infrastructure, poor pest and disease management, and most critically, widespread use of open-pollinated varieties (OPVs) with inferior genetic potential (Khar and Singh, 2020; Raghu, 2024). The slow adoption of F_1 hybrids despite their demonstrated superiority in yield, bulb uniformity, genetic purity, stress resistance, and mechanisation suitability further limits productivity gains (Veere Gowda et al., 2002). Unlike India, major onion-producing countries have successfully transitioned to hybrid varieties, resulting in substantial yield improvements (Gabelman, 1974; Khar et al., 2022). To bridge this gap, India must invest in robust hybrid breeding programmes and seed distribution systems. Embracing modern breeding technologies is essential to enhance onion productivity and strengthen India's competitiveness in the global market.

A. Hybrid Onion Breeding: Key Prerequisites and Considerations

The development of onion hybrids hinges on exploiting heterosis from genetically diverse parental lines, with significant gains observed in traits like bulb yield and quality (Aghora and Pathak, 1991). However, hybrid viability depends on breeding mechanisms such as pollination systems, seed multiplication, and genetic control. Onions, being insect-pollinated and naturally cross-pollinated, are uniquely suited for hybrid breeding. The presence of stable cytoplasmic male sterility (CMS) systems further enhances hybrid seed production efficiency (Pathak, 1997; Singh and Khar, 2021), eliminating the need for manual emasculation often impractical due to the small, asynchronous onion flowers.

Key Requirements for Successful Onion Hybrid Breeding:

1. Genetically Diverse and Superior Parental Lines

Effective hybrid performance relies on using highly homozygous and genetically diverse parental lines. Greater divergence between female and male lines increases heterosis and ensures hybrid uniformity.

2. CMS-Based Hybrid Seed Production

Hybrid seed production in onion uses a three-line CMS system:

- **A-line** (male sterile): Seed parent ensuring hybrid purity
- **B-line** (maintainer): Maintains male sterility in A-line
- **C-line** (male parent): Provides viable pollen for hybrid seed production

CMS eliminates manual emasculation, enabling efficient and large-scale hybrid seed production with minimal contamination risk.

B. Inbred Parental Lines: Backbone of High-Performing Onion Hybrids

Inbred lines are critical to onion hybrid development. Achieved through successive self-pollination, these lines must exhibit:

- High homozygosity and genetic purity
- Superior per se performance in yield, bulb traits, and storability
- Uniformity in flowering and plant type
- High seed yield potential
- Strong general and specific combining ability
- Resistance to major biotic and abiotic stresses
- Wide environmental adaptability

Genetically stable and diverse inbred lines facilitate robust, high-yielding hybrid combinations across environments.

C. Challenges in Developing True Inbred Lines in Onion

Onion's protandrous and cross-pollinated nature leads to a high genetic load many recessive deleterious alleles remain hidden in heterozygous states. Selfing exposes these alleles, causing severe inbreeding depression after just 2-3 generations. Consequences include:

- Reduced plant vigour and bulb yield
- Poor seed set and increased thick-neck bulbs
- Decline in disease resistance and stress tolerance

Moreover, as a biennial crop, onions require at least two years per generation cycle, prolonging breeding timelines. These biological constraints significantly hinder the development of true inbred lines necessary for hybrid breeding.

Solutions: Integrating modern tools like doubled haploid technology, marker-assisted selection (MAS), and speed breeding can accelerate inbred line development and mitigate inbreeding depression.

D. Processes and Methods for Inbred Line Development in Onion

Hybrid development in onion follows five key steps:

1. Inbred line development
2. Evaluation of inbreds
3. Creation of hybrid combinations
4. Hybrid evaluation and selection
5. Large-scale seed production

The pedigree method is commonly used: selection and selfing over 6-7 generations to reach near-homozygosity. However, in onions, this often leads to excessive inbreeding depression. Reports indicate:

- 64% bulb yield in selfed lines vs. open-pollinated
- 12-day maturity delay
- Increase in thick-neck bulbs from 2% to 12%
- Reduced survival (50%) and seed set (70%) after three selfed generations (Jones and Mann, 1963; Dowker and Fennel, 1981)

Since inbreeding effects vary by genotype (Khan et al., 2001), breeders are encouraged to use modified approaches such as:

- Modified pedigree methods
- Bulk pedigree
- Half-sib or full-sib mating after early generations

These methods maintain a balance between increasing homozygosity and minimizing inbreeding depression, improving the success rate of onion inbred line development.

1. Modified Pedigree or Bulk Pedigree Method for Inbred Line Development in Onion

The modified pedigree or bulk pedigree breeding method offers a practical approach for developing inbred lines in onion, balancing the need for homozygosity with the need to avoid severe inbreeding depression. The methodology combines individual plant selection with population-level bulking and controlled mating to produce uniform, vigorous inbred lines over successive generations.

Stepwise Procedure:

a) Initial Selection and Selfing

- The base population may originate from an open-pollinated variety (OPV) or from segregating progeny of a cross between two distinct parental lines. In the first year:
- Superior bulbs are selected based on phenotypic traits or general combining ability (GCA) through testcrossing (using a wide genetic base tester).
- Each selected bulb is self-pollinated, and seeds are harvested separately from each plant.

b) Evaluation of Progeny Rows *(Second bulb crop year / Third overall year)*

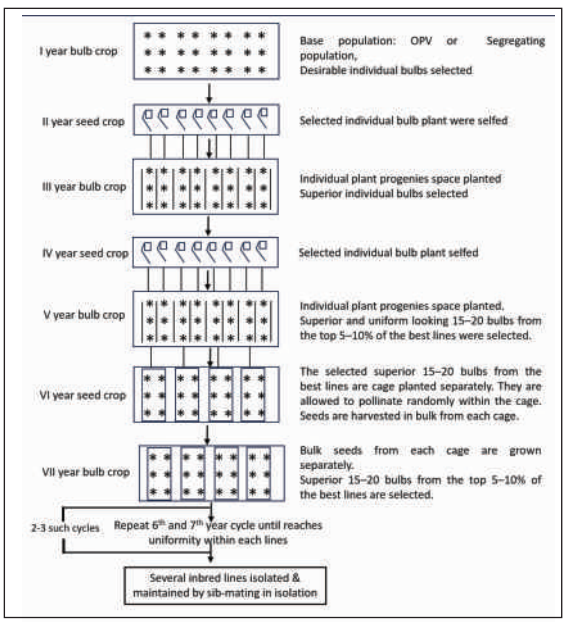
- Around 30-40 bulbs per selfed line are space-planted.
- Progeny rows are evaluated, and superior bulbs are selected from high-performing lines.
- Poor progenies are eliminated via negative selection.
- Seeds are either harvested in bulk from top-performing lines or, if no clear superiority is observed, superior individual plants are selfed again.

c) Advanced Selection and Cage Crossing *(Fifth year overall)*

- Progeny rows are grown again, and 15–20 morphologically similar and superior bulbs are selected per line.
- Approximately 5-10% of the top-performing lines are selected.
- These selected bulbs are cage-pollinated to facilitate random crossing among selected siblings.
- Seeds are harvested in bulk per group.

d) Repeated Selection and Cage Mating

- Bulks from each cage-crossed progeny are planted and evaluated for morphological uniformity and trait performance.
- Selected bulbs are again cage-pollinated, and the cycle is repeated for 2–3 generations until uniformity and stability are achieved.



e) Line Stabilization and Maintenance

- Once stable, the inbred lines are maintained through controlled sib-mating in isolation to preserve vigor while maintaining genetic purity.
- These lines are then subjected to further evaluation for hybrid development.

Fig 1. Schematic representation of the modified pedigree or bulk-pedigree method for developing inbred lines in onion

Conventional breeding methods, with slight modifications, remain the most widely employed techniques for developing high-performing parental and breeding lines in onion. For instance, Kim et al. (2007) utilized traditional breeding strategies to develop red, mild-tasting, and less pungent onion cultivars. In their study, the mild yellow onion cultivar 'Texas Grano 502' was crossed with the red onion cultivar 'Cardinal'. Through successive generations of phenotypic selection for bulb colour, the breeding line '43108' was developed. Segregating populations yielded yellow and pink-coloured bulbs, from which red and pink bulbs were selected, while yellow bulbs were discarded based on visual phenotypic traits in each generation.

The term "random mating" in this context refers to outcrossing among selected individuals within a line. This approach is commonly used in onion breeding programs to maintain breeding lines through random mating among phenotypically similar plants, thereby mitigating the adverse effects of severe inbreeding depression (Kim et al., 2007).

Similarly, Pathak and Gowda (1994) successfully applied conventional breeding methods in India during the 1980s to develop four distinct inbred male parental lines (C-lines): Sl 13-1-1-1, Sl 14-1-1-1, Sl 11-1-1-1, and NER-1-1. This foundational work enabled the development and release of two cytoplasmic male sterile (CMS)-based hybrid varieties Arka Kirthiman and Arka Lalima-derived from the inbred lines Sl 13-1-1-1 and Sl 14-1-1-1, respectively. These innovations have significantly advanced hybrid onion cultivation in India (Veere Gowda et al., 1998).

Challenges Associated with Conventional Inbred Line Development in Onion

Despite their widespread use, conventional breeding methods-such as the modified pedigree or bulk pedigree approaches-pose several significant challenges in onion breeding:

1. **Severe Inbreeding Depression**: Repeated selfing leads to reduced plant vigor, smaller bulb size, decreased fertility, and low seed set, hindering generational advancement.
2. **Biennial Life Cycle**: Onion's biennial nature, requiring separate bulb and seed production phases, slows down breeding progress. Achieving homozygosity through successive selfing may require 8–10 years or more.
3. **Labor-Intensive Selfing and Isolation**: Manual self-pollination or controlled sib-mating is time-consuming and labour-intensive. Physical isolation or pollination cages are necessary to prevent cross-pollination.
4. **Low Seed Set in Inbred Lines**: Inbreeding often reduces fertility, leading to poor seed production and difficulty in maintaining inbred lines.
5. **Genetic Drift and Selection Pressure**: Small effective population sizes during selfing increase the risk of genetic drift and unintended selection, reducing genetic diversity.
6. **Bulb Heterogeneity and Segregation**: Early-generation heterozygosity results in significant variation in bulb size, shape, and colon, complicating selection for uniformity.

7. **Increased Disease Susceptibility**: Reduced genetic variability in inbred lines often leads to heightened susceptibility to diseases, negatively impacting field performance and line maintenance.

2. Backcross Method for CMS Diversification in Onion

The backcross method is a widely utilized breeding strategy for diversifying cytoplasmic male sterility (CMS) sources in onion. This approach involves using a male-sterile line (A-line), which carries a specific sterile cytoplasm, as the female (donor) parent in a systematic backcrossing program. The recurrent parent characterized by desirable agronomic traits and a normal (fertile) cytoplasm is employed as the male parent in both the initial cross and all subsequent backcross generations.

Throughout the backcrossing process, the sterile cytoplasm of the donor line is retained, while the nuclear genome of the recurrent parent is progressively recovered. After approximately six to eight backcross generations, the progeny will possess the nuclear genetic background of the recurrent parent and the sterile cytoplasm of the donor. This results in the development of a new A-line (male-sterile line) that mirrors the agronomic performance of the recurrent parent but remains male-sterile due to the donor cytoplasm.

Simultaneously, the original recurrent parent serves as the B-line (maintainer line), as it shares an identical nuclear genome with the derived A-line but retains fertile cytoplasm. The B-line plays a critical role in maintaining the A-line through regular crosses, ensuring the continued propagation of male-sterile lines for hybrid seed production.

Table 1. Diversification of cytoplasmic male sterility (CMS) in onion using the backcross breeding method

Generation	Cross	Resulting Genotype	Nuclear Genome Contribution from B-line (%)
Initial Cross	S msms (A-line) × N msms (B-line)	F_1: S msms	50.00%
Bc_1	S msms × N msms	Bc_1: S msms	75.00%
Bc_2	S msms × N msms	Bc_2: S msms	87.50%
Bc_3	S msms × N msms	Bc_3: S msms	93.75%
Bc_4	S msms × N msms	Bc_4: S msms	96.88%
Bc_5	S msms × N msms	Bc_5: S msms	98.44%

Note: S = sterile cytoplasm; N = normal (fertile) cytoplasm; msms = recessive nuclear male-sterility gene. After five backcross generations, the resulting male-sterile (A-line) possesses the sterile cytoplasm of the donor parent and ~98.44% of the nuclear genome of the recurrent male-fertile (B-line) parent, effectively creating a new CMS line genetically similar to the B-line.

Schematic Plan for Diversifying Cytoplasmic Male Sterility (CMS) in Onion

Adapted from Pike (1986)

Year	Procedure
1	Grow and select 100 bulbs based on desirable traits. Store and replant them. Simultaneously, maintain a supply of male-sterile bulbs to be used in test crosses.
2	Perform self-pollination of the selected bulbs. In parallel, make test crosses using a known male-sterile (A-line) as the female parent.
3	Grow progeny from both selfed bulbs and the resulting F_1 test crosses. Evaluate and select superior progeny rows; discard underperforming rows along with their corresponding F_1 pairs. Store bulbs from the selected lines.
4	Plant selected bulbs for seed production. Assess sterility expression in F_1 progeny. If an F_1 line exhibits 100% sterility, self the selected bulbs and make a backcross to the F_1. Discard any lines showing fertility in the F_1 generation.
5	Grow male-sterile (A-line) and fertile (B-line) pairs. Continue selection within the B-line progeny for agronomic performance. Retain the best bulbs from the A-line for further backcrossing.
6	Self-pollinate selected B-line progenies and continue backcrossing to the A-line (sterile) side of the pair.
7	Grow bulbs from the selected pairs and perform final selections based on the performance of the B-line side.
8	Mass propagate the B-line using 10–20 selected bulbs in isolation cages. Concurrently, continue backcrossing to the A-line side.
9-12	Initiate seed-to-seed propagation. Repeat the procedure outlined in Year 8 to complete up to five backcross generations. By this stage, stable A- and B-line pairs should be established. Begin the evaluation and testing of hybrid combinations derived from these lines.

Pathak and Gowda (1994) effectively applied the backcross breeding method in the 1980s to diversify sterile cytoplasm in short-day onion genotypes in India. Male sterility was first identified in the variety *Bombay White Globe* (IIHR 20) (Pathak et al. 1980), and this trait was subsequently transferred into several breeding lines with diverse genetic backgrounds through successive backcrossing. This effort resulted in the development of six cytoplasmic male-sterile (CMS) lines-MS1, MS8, MS11, MS39, MS48, and MS65-which were later utilized in generating a range of F_1 hybrid combinations.

These CMS lines (A-lines) were maintained by crossing with their corresponding maintainer lines (B-lines) under controlled conditions using large isolation cages to prevent cross-contamination. The

resulting seed production system ensured the stable propagation of male sterility across generations.

Subsequent heterosis and combining ability studies involving the MS lines and inbred parental lines led to the identification of promising hybrid combinations. Among these, Arka Kirthiman and Arka Lalima emerged as successful CMS-based hybrids (Veere Gowda et al., 1998). Notably, Aghora and Pathak (1991) reported a heterosis of up to 28.47% in bulb yield over the best commercial check variety, demonstrating the effectiveness of CMS-based hybrid breeding in enhancing onion productivity.

Fig. 2. India's first commercial onion hybrids Arka Kirthiman and Arka Lalima developed using cytoplasmic male-sterile (CMS) lines derived through backcross breeding. These hybrids marked a significant milestone in Indian onion breeding, offering enhanced bulb yield, uniformity, and improved adaptation under short-day conditions.

3. Marker-Assisted Backcross Breeding (MABC) Method for Rapid CMS Conversion in Onion

Onion is a biennial crop, requiring two growing seasons to complete its life cycle, which significantly slows conventional breeding progress. Traditional backcross breeding for cytoplasmic male sterility (CMS) conversion can take up to 10–12 years to introgress sterile cytoplasm into diverse genetic backgrounds. In contrast, Marker-Assisted Backcross Breeding (MABC) offers a significantly accelerated alternative, reducing the time required to 3-4 years.

MABC combines traditional breeding approaches with molecular marker technologies, allowing breeders to efficiently and precisely introgress CMS into elite cultivars while maintaining the desirable nuclear genome of the recurrent parent. The integration of markers allows accurate selection at each generation and minimizes the reliance on labor-intensive phenotypic screening, which is often influenced by environmental variability.

Key Steps in Marker-Assisted Backcross Breeding:

1. Foreground Selection: Identify and select plants carrying the CMS cytoplasm using cytoplasm-specific molecular markers.
2. Background Selection: Use genome-wide molecular markers (e.g., SSRs, SNPs) to identify individuals with the highest recovery of the recurrent parent genome.
3. Recombinant Selection: Eliminate undesirable donor genomic segments, especially those linked to deleterious traits, to produce lines with minimal linkage drag.

This process enables the rapid development of elite CMS lines (A-lines) that retain the agronomic performance of the recurrent parent while incorporating the desired sterile cytoplasm.

Application of Molecular Markers in Onion CMS Breeding:

Several molecular markers have been developed for cytoplasmic differentiation in onion:

- Havey (1995), Sato (1998) and Von Kohn et al. (2013) identified markers that distinguish between normal (N) and sterile (S) cytoplasm.
- Kim et al. (2009) further refined this by classifying all three major cytoplasm types: S, T and N.
- Khar et al. (2022) reported the use of PCR-based markers such as *cob* and *MKFR* for differentiating cytoplasmic types (S, T, N) in onion breeding populations.

These markers have revolutionized CMS-based hybrid breeding by enabling precise cytoplasm identification and ensuring efficient CMS line development and maintenance, thereby enhancing the reliability and scalability of hybrid seed production in onion.

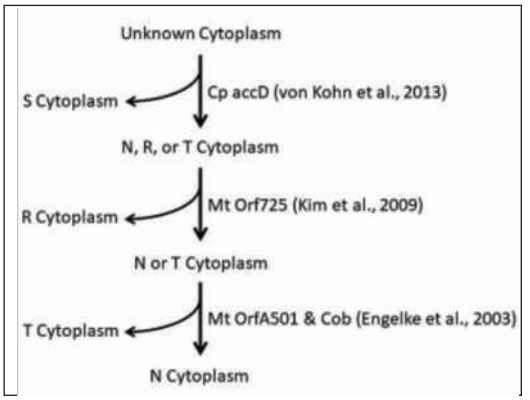


Fig. 3. Molecular markers distinguishing onion cytoplasm (Source: Kim et al., 2009).

4. Development of Inbred Lines Using Doubled Haploid (DH) Technology in Onion

The application of doubled haploid (DH) technology provides a powerful and efficient strategy for the rapid development of completely homozygous inbred lines in onion (*Allium cepa* L.), a crop known for its biennial life cycle and high degree of heterozygosity. Traditional inbreeding approaches in onion such as selfing and sib-mating are hampered by severe inbreeding depression, long generation intervals, and the requirement of multiple cycles to achieve homozygosity. In contrast, DH technology can produce fully homozygous lines in a single generation, thereby significantly accelerating onion breeding programs.

Gynogenesis: The Preferred Pathway in Onion

In onion, gynogenic haploid induction is the most effective method for DH production, as androgenic approaches (anther or microspore culture) have proven largely ineffective (Belwal et al., 2024). Gynogenesis involves the in vitro culture of unfertilized ovules or ovaries, leading to the formation of haploid embryos, which are subsequently regenerated into haploid plantlets under sterile, controlled conditions (Poornima et al., 2024).

Chromosome Doubling and DH Line Development

Once haploid plantlets are obtained, chromosome doubling is induced using anti-mitotic agents such as colchicine or oryzalin. This process restores fertility and results in fully homozygous doubled haploid (DH) plants, which are genetically fixed at all loci. These DH lines are then rigorously evaluated for key agronomic traits such as bulb shape, size, colour, storability and disease resistance. Superior lines are selected for potential use as parental lines in hybrid breeding or for direct cultivar release.

Process of DH Line Development in Onion

1. Gynogenic Induction

a. Unopened flower buds are cultured *in vitro* to induce haploid embryo formation from unfertilized ovules.

b. The success of gynogenesis is highly genotype-dependent, with varying responsiveness among onion genotypes (Poornima et al., 2024).

2. Regeneration of Haploid Plants

a. Haploid embryos are regenerated into plantlets under controlled tissue culture conditions.

b. Ploidy levels are confirmed, often using flow cytometry or chromosome counting, to distinguish haploids from diploids.

3. Chromosome Doubling

a. Chromosome number in haploid plants is doubled using anti-mitotic agents such as colchicine or oryzalin.

b. This step restores fertility and results in fully homozygous DH plants.

4. Evaluation and Selection

a. DH lines are evaluated for critical traits including bulb size, shape, colour, storability, and biotic/abiotic stress tolerance.

b. Promising lines are selected for hybrid development, marker-assisted selection, or direct cultivar release.

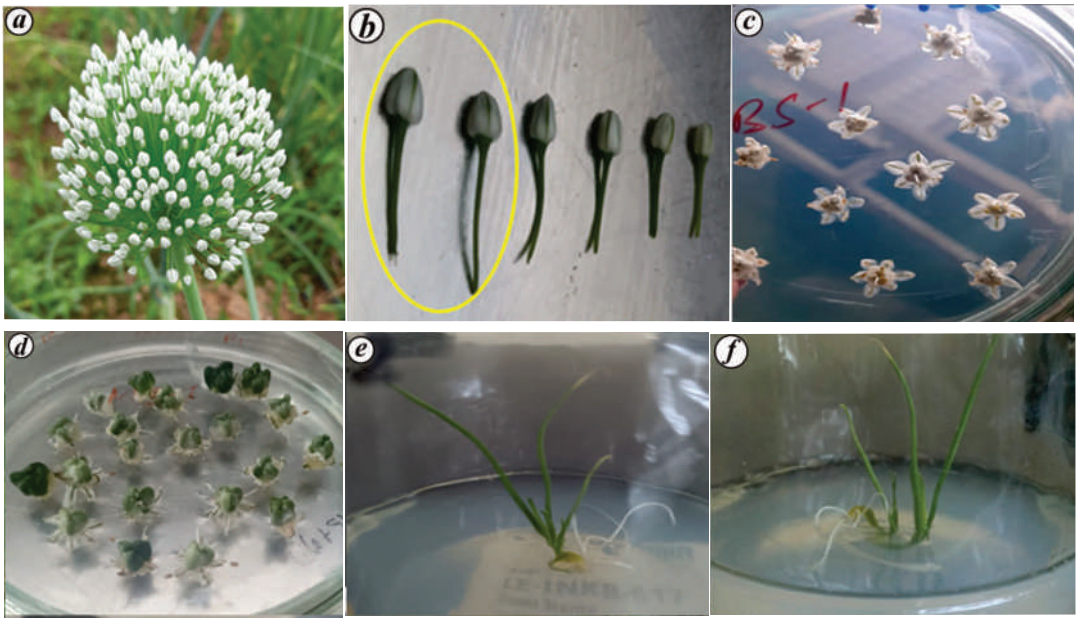


Fig. 4. Onion doubled haploid production. a, Right stage of flower for bud picking; b, Optimum flower bud size (3.5-4.5 mm); c, Flower buds after two weeks of inoculation; d, Callus formation in flower buds; e and f, Shooting and rooting of the flower buds forming plantlets (Source: Poornima et al., 2024).

5. Fast Tracking Inbred Line Development in Onion Using Speed Breeding

Onion is a biennial crop, requiring two growing seasons-one for bulb formation and the other for seed production which inherently prolongs its breeding cycle. Traditional methods for developing inbred lines often take 10-12 years due to this extended life cycle and the crop's susceptibility to inbreeding depression. In this context, speed breeding emerges as a powerful strategy to accelerate genetic gain and reduce the breeding timeline in onion. Speed breeding involves the manipulation of environmental conditions such as photoperiod, temperature, and light intensity-to shorten the duration between generations. When integrated with other advanced breeding tools such as doubled haploid (DH) technology and molecular markers, speed breeding has the potential to revolutionize onion improvement programs.

Key Speed Breeding Techniques in Onion

1. **Controlled Environment Cultivation:** The use of growth chambers or greenhouses with optimized environmental conditions-particularly extended photoperiods (e.g., 22-hour light exposure) and regulated temperatures can accelerate bulb initiation and maturity. Research indicates that under speed breeding conditions, bulb development can be achieved in as little as

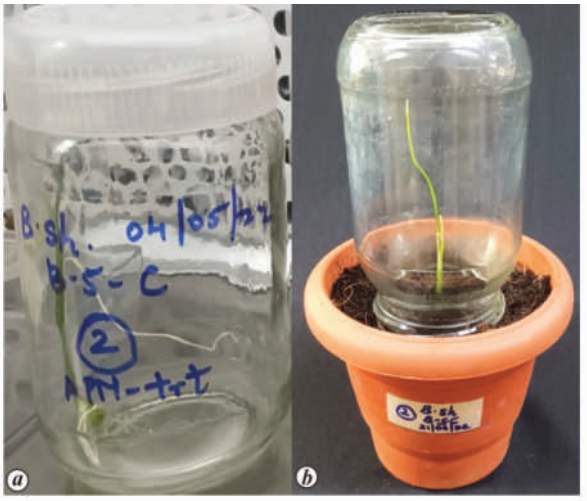


Fig. 5. a, Haploid plantlets treated with ami-prophos methyl (APM) for chromosome doubling. b, Hardening of haploid plantlets after APM treatment (Source: Poornima et al., 2024).

45 days, with plant maturity occurring in approximately 80 days, compared to the 5–6 months typically required under open-field conditions (Brewster, 2008; Khosa and Dhatt, 2020).

2. **Artificial Vernalization and Dormancy Disruption:** Onions require vernalization (exposure to low temperatures) to induce flowering. Artificial vernalization involves exposing bulbs or seedlings to controlled cold treatments to trigger early flowering. Additionally, chemical treatments such as hydrogen peroxide have been used to break bulb dormancy, promoting uniform root emergence and advancing flowering (D'Angelo and Goldman, 2019).
3. **Integration with Molecular Tools:** The recent sequencing of the onion genome has enabled breeders to identify molecular markers associated with critical traits such as disease resistance, bulb shape, and storability. Combining speed breeding with marker-assisted selection (MAS) and genomic selection ensures rapid, accurate identification and advancement of desirable genotypes.
4. **Off-Season and Double Cropping:** Utilizing contrasting agroclimatic regions or greenhouses for off-season nurseries allows the cultivation of multiple generations per year. For instance, in regions like Bengaluru, Karnataka, it is feasible to produce a bulb crop during the *kharif* season and a seed crop during the *rabi* season, effectively doubling annual breeding throughput.
5. **Use of Plant Growth Regulators:** Growth regulators such as gibberellic acid (GA_3) can promote early bolting and flowering, thereby shorten the vegetative phase, and expedite seed production. This chemical induction can be particularly useful in genotypes that exhibit late flowering under normal conditions.

Challenges and Considerations

While speed breeding holds significant promise, several limitations must be addressed for successful implementation:

- **Genotypic Variability:** Different onion genotypes may respond variably to altered environmental cues, necessitating the development of genotype-specific protocols.
- **Resource Intensiveness:** The establishment and maintenance of controlled environments, coupled with the integration of biotechnological tools, require substantial investment, technical expertise and infrastructural support.

E. Hybrid Seed Production in Onion

Hybrid seed production in onion is a critical step in commercial onion breeding programs, aimed at harnessing heterosis (hybrid vigor) for enhanced productivity, uniformity, and quality. F_1 hybrids typically outperform open-pollinated varieties in terms of bulb yield, size, shape, storability, disease resistance, and adaptability across environments.

1. Flower Morphology in Onion

Fig. 6. Onion inflorescence Before (A) and after anthesis (B)

Onion (*Allium cepa* L.) is an outbreeding, insect-pollinated crop, primarily due to its floral morphology and protandrous nature. The flowers are small, white and borne in a compound umbel, each comprising 50 to 2000 individual florets. Each floret possesses:

- Six perianth segments,
- Six stamens,
- One pistil formed by three fused carpels with a trilocular ovary,
- Two ovules per locule (maximum of six seeds per flower) and
- Nectaries that facilitate pollinator attraction.

Protandry ensures that the anthers mature 3-4 days prior to stigma receptivity, encouraging cross-pollination. However, overlapping anthesis within umbels can result in up to 5% self-pollination. Anthesis typically begins early morning (6-7 AM) and extends until 9-10 AM, with anther dehiscence continuing through the day, peaking between 9:30 AM and 5:00 PM. Pollen fertility is highest on the day of anthesis, while stigma receptivity peaks 3-4 days later.

The fruit is a capsule with a natural shattering habit, necessitating timely harvest to prevent seed loss.

2. Utilization of Male Sterility in Hybrid Onion Seed Production

Manual emasculation is impractical in onion due to the small floral size and large number of florets per umbel. Hence, cytoplasmic male sterility (CMS) is employed as a reliable system for F_1 hybrid seed production.

The first report of CMS in onion was by Jones and Emsweller (1936), who identified male sterility in the cultivar *Italian Red*. Further studies by Jones and Clarke (1943) elucidated that male sterility is controlled by sterile (S) cytoplasm interacting with a recessive nuclear gene (*ms*). The male sterility is associated with tapetal degeneration rather than meiotic abnormalities (Saraswathi and Veere Gowda, 2006).

- S msms: Male sterile (A-line)
- N msms: Male fertile (B-line or maintainer)
- Cytoplasmic inheritance being maternal, all progeny from S msms × N msms cross will inherit sterile cytoplasm and be male sterile.

3. Development of A, B, and C Lines

Hybrid onion breeding involves the development of three distinct parental lines:

- A-line (CMS line): Genotype S msms; incapable of producing viable pollen; used as the female parent.
- B-line (Maintainer line): Genotype N msms; genetically isogenic to the A-line except for cytoplasm; used to maintain CMS lines through backcrossing.
- C-line (Pollen parent): Male fertile, genetically diverse, selected for combining ability and agronomic performance; used to pollinate A-line for hybrid seed production.

Repeated backcrossing (5–6 generations) between the A- and B-lines ensures near-isogenicity, critical for uniform hybrid seed quality.

4. Techniques of Hybrid Seed Production

Hybrid seed production is executed under controlled field conditions or in isolated environments where:

- CMS (A-line) and pollen parent (C-line) are planted in specific ratios (e.g., 2:1 or 3:1).
- Synchrony in flowering between A- and C-lines is essential for optimal pollen transfer.
- Insect pollinators, particularly honeybees, are critical for successful pollination due to the outcrossing nature of the crop.
- Only A-line seed umbels are harvested as they contain the F_1 hybrid seed.

Due to climatic constraints, bulb production and seed production are often conducted in separate locations. For instance, bulb multiplication may occur in one season/location, while seed production takes place under optimal flowering conditions elsewhere.

5. Climate Requirements for Hybrid Seed Production

Onion hybrid seed production requires well-defined climatic conditions:

- Vegetative phase: Mild, cool temperatures (~20°C) with low humidity.
- Reproductive phase: Gradual rise in temperature to 25–30°C promotes flowering and seed development.
- Pollination stage: Clear, sunny weather is ideal to promote insect activity.
- Post-pollination (maturity, curing, threshing): Dry conditions are critical to minimize seed loss due to natural fruit shattering.

Excessive rain, dew or fog during flowering and seed development stages increases susceptibility to diseases such as purple blotch, *Stemphylium* blight and downy mildew.

6. Planting Season for Hybrid Seed Production in Onion

a. Northern and Eastern India

- Mother bulb production: October to January
- Bulb storage: February to September
- Bulb planting for seed production: October
- Seed harvesting: Late January to February

b. Southern India (e.g., Bengaluru, Karnataka)

- The region experiences mild climate (avg. temp. ~27°C) and moderate *kharif* rainfall, allowing for two annual onion cycles.
- Mother bulb production: June to September
- Bulb storage: October
- Bulb planting for seed production: November
- Seed harvesting: March

Note: Although onion bulbs do not exhibit a true dormancy period, proper post-harvest curing and drying are essential to ensure sprouting uniformity and enhance subsequent flowering.

7. Soil Requirements

- Mother bulb production: Prefers light sandy loam soils with excellent drainage and aeration for proper bulb enlargement.
- Seed production: More suited to heavier, cooler soils that offer good water-holding capacity and reduce lodging during reproductive stages.

8. Land Preparation

- Prepare the soil to a fine tilth through ploughing, harrowing and disking.

- Incorporate 25 tons/ha of well-decomposed organic manure during the final land preparation.
- Form ridges and furrows spaced 30 cm apart, organizing plots into segments of approximately 3 meters in length.

9. Fertilizer Application

- Basal dose: Apply 75:80:50 kg NPK/ha into furrows and mix well into the soil.
- Topdressing of 75 kg N/ha in two equal splits:
  First split: 30 days after transplanting
  Second split: 45 days after transplanting

10. Production of Mother Bulbs

Mother bulbs for both A-line (female/CMS) and C-line (male/fertile) parents are cultivated using conventional agronomic practices, similar to commercial cultivars. To prevent genetic contamination:

- Use colour-coded labels to mark plots and distinguish between A- and C-lines.
- Maintain strict identity preservation during harvest, curing and storage.

11. Storage of Mother Bulbs

- Cure harvested bulbs adequately to remove excess moisture.
- Store selected bulbs at 11-12°C and 70-75% relative humidity, which:
  Promotes uniform and early flowering
  Enhances seed setting and overall seed yield
- Higher temperatures during storage may delay or suppress flowering.

12. Selection of Mother Bulbs

- Choose medium-sized, uniform, true-to-type bulbs.
- Slice off the top one-third of each bulb to: examine internal bulb quality, detect disease or physiological disorders
- Prioritize single-centered bulbs, as they ensure uniform flowering and better seed set compared to double-centered bulbs.

13. Planting of Bulbs for Seed Production

- Plant A- and C-lines in alternating rows with a common female-to-male ratio of 4:1 or 8:2, depending on the hybrid's pollination requirement.
- Cut the top third of each bulb and treat with 5% Blitox paste to reduce rotting.
- After treatment, shade-dry the bulbs, then plant halfway into the ridge at 30 × 15 cm spacing, ensuring the cut surface is partially exposed above ground.
- Maintain uniform soil moisture until sprouting is complete.

Spacing Consideration:

- Closer spacing (e.g., 30 × 10 cm) increases seed yield but also raises disease pressure.
- Row-to-row spacing: 30 cm
- Plant-to-plant spacing: Adjust between 10-15 cm depending on disease risk and hybrid vigour.

Fig. 7. Selection of desirable-sized mother bulbs, with the top one-third portion cut and treated with 5% Blitox paste (A); planting of treated mother bulbs in the field (B).

14. Nutritional Management

Maintaining optimal nutrient balance is essential for achieving high seed yields and quality in onion:

- Nitrogen (N): Critical during early vegetative stages and until seed stalk differentiation. Nitrogen deficiency can limit leaf area and plant vigour, impacting flowering potential.
- Phosphorus (P) and Potassium (K): Required in larger quantities during reproductive stages to support flowering, seed setting and seed development.
- Micronutrients: Application of boron, molybdenum, zinc and manganese has been shown to improve both seed yield and quality. These elements support pollen viability, ovule development and seed maturation.

15. Irrigation Management

- Maintain optimal soil moisture throughout the crop duration, particularly during flower initiation and seed filling stages.
- Avoid over-irrigation, which can:
    - Promote soil-borne pathogens and basal rots.
    - Cause lodging of seed stalks.
    - Lead to mixing of lines, affecting seed purity and quality.

16. Flowering Synchrony

Achieving synchronized flowering between A (CMS) and C (pollinator) lines is vital:

- The C line should flower slightly earlier than the A line to ensure pollen availability throughout the A-line's flowering period.
- If natural synchrony is lacking, adjust:
    - Planting dates
    - Line selection with known synchrony traits

This ensures optimal cross-pollination and hybrid seed set.

17. Use of Pollinators

Efficient pollination is critical in onion hybrid seed production:

- Honeybees (*Apis* spp.) are the most reliable and effective pollinators.
- Recommended density: 3-4 beehives per acre.
- Promote a pollinator-friendly environment by avoiding pesticide sprays during flowering.

18. Roguing

To maintain genetic purity, roguing is conducted to remove:

- Male-fertile plants in the A line.
- Off-types and phenotypic deviants.

Timing:

- Best performed in the early morning, before anther dehiscence, when pollen is visible on fertile anthers.
- Method: Entire rogue plants should be uprooted, and inflorescences destroyed or buried-never discarded openly, as they can continue shedding pollen for weeks.

19. Use of Morphological Markers

Morphological markers help detect unwanted fertile or off-type plants in the A line:

Marker	Trait	Use
Perianth Colour	White (recessive) vs Green	A & B lines fixed for white; rogue out green-perianth plants early
Foliage Type	Glossy (recessive)	Glossy A-line allows roguing of non-glossy outcrosses
Anther Traits	Colour and length	Recessive in A & B lines; dominant in C line for easy post-flowering roguing

20. Isolation Requirements

To maintain genetic purity:

- 2 km isolation for varieties of the same bulb colour
- 3 km isolation for varieties of different bulb colours

Isolation prevents unwanted cross-pollination with nearby onion varieties.

21. Seed Harvesting

a. Seed Maturity Index

- Seed stalks mature in 110-120 days after planting.
- Indicators of maturity:
  Seed stalks turn from green to yellow
  Umbels begin to yellow, with central capsules opening
- Staggered harvesting prevents seed loss due to capsule shattering.

b. Harvest Method

- If C-line seeds are not required, destroy their umbels after pollination using mowing or disking.
- If seeds from both lines are needed:
  Harvest A-line (hybrid seeds) first.
  Separate intermixed plants if lodging has occurred.
- Post-harvest, cure umbels in shade to reduce seed moisture before threshing.

22. Threshing and Milling

- Manual methods (crushing, flailing) or machine threshers can be used.
- Be gentle-onion seeds have surface-located embryos and are susceptible to mechanical damage.
- Remove plant debris and rogue seeds using winnowing, sieving or gravity separators.

23. Seed Yield

- Average hybrid seed yield: 350-500 kg/ha
- Under ideal conditions (effective pollination, high male sterility), yields can exceed 500 kg/ha

24. Seed Storage

- Dry seeds to 6-8% moisture, preferably down to 5%.
- Store in airtight containers under cool, dry, and well-ventilated conditions.
- Ideal storage:
  Temperature: ~6°C
  Relative Humidity: ~75%
- Onion seeds are short-lived, hydroscopic, and prone to viability loss; hence, storage under controlled conditions is critical to maintain germination.

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Performance and Economic Comparison of F_1 Hybrids and Open-Pollinated Varieties (OPVs) in Onion

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Abstract

Onion is a crop of considerable nutritional, economic, and political significance in India, which contributes more than 30% of global onion production. Despite its prominence, the sector remains highly vulnerable to recurrent price volatility driven by production fluctuations, climatic stresses, substantial post-harvest losses, and persistent supply-demand imbalances. Relatively low productivity compared with major onion-producing countries is largely attributable to the continued predominance of OPVs, short-day growing conditions and inherent constraints in conventional breeding. Although F_1 hybrids involve higher seed costs, they offer clear advantages in terms of yield potential, uniformity, bulb quality and marketability, whereas OPVs continue to be widely adopted due to their lower seed cost, broader adaptability, and compatibility with farmer seed-saving practices. This review critically evaluates the agronomic performance and economic returns of F_1 onion hybrids relative to OPVs under diverse Indian agro-climatic conditions. It highlights the role of breeding strategies, climatic challenges, and technological interventions in improving productivity and profitability. The study underscores the need for balanced varietal deployment, strengthened public-sector hybrid development and integration of modern breeding approaches to ensure sustainable onion production, price stability and enhanced farmer income in India.

Introduction

Onion (*Allium cepa* L.), a widely cultivated and consumed spicy-vegetable herb crop belonging to the genus *Allium* and the family Alliaceae, holds significant global importance for its pungent flavor and extensive culinary applications (Brewster, 2008). It is consumed both as a vegetable (green onions and mature bulbs) in fresh culinary preparations and as a spice-condiment in processed forms such as powder and dried flakes, enhancing the flavor of diverse cuisines (Sharma et al. 2020; Teshika et al. 2018). Beyond its culinary value, onion is highly esteemed for its nutraceutical properties, medicinal benefits, and ethno-pharmacological applications, making it a valuable crop in both dietary and therapeutic practices (Chakraborty et al. 2022; Singh et al. 2023; Taleghani et al. 2024).

Onion is recognized as a unique and versatile TOP crop in India due to its politically sensitive and economically significant nature, making it one of the most closely monitored agricultural commodities (Premi and Premi, 2017; Kalamkar and Sharma, 2019). As one of the largest and leading producers of bulb onions, India accounts for over 30% of the world's total onion production (FAOSTAT, 2023). However, in recent years, the country has experienced recurring onion crises, characterized by soaring prices and supply chain disruptions, primarily driven by weather-related factors and rising export demand. This ongoing supply-demand imbalance underscores the crop's critical agricultural and economic importance, with frequent fluctuations significantly impacting both domestic and global markets (Gedam et al. 2021; Godke et al. 2018; Mahajan and Gupta, 2015; Kavalgi et al. 2025; Economic Survey, 2024-25).

Area, production, consumption, export and import statistics:

In 2024, India produced 24.26 million metric tons of onions from an area of 1.94 million hectares. However, the market faced a significant onion crisis due to supply-demand imbalances. To address this, onion production in 2025 is projected to increase to 28.87 million metric tons, covering an expanded area of 3.29 million hectares, representing a nearly 19% rise in production compared to the previous year (PIB, 2024). However, domestic onion consumption is estimated at approximately 20 million metric tons annually (>19.36 million tons), and this figure continues to grow over time. Among all states and Union Territories, Uttar Pradesh (UP), with the largest population, records the highest onion consumption at nearly 2.1 million tons per year, followed by Bihar (2.08 million metric tons) and Maharashtra (1.71 million metric tons) (Economic Survey, 2024-25). According to FAOSTAT (2021), India's per capita onion consumption reached an all-time high of 16.3 kg per year in 2021 a 1.3% increase from the previous year-rising from 14.7 kg in 2017. This marks a substantial increase from the lowest recorded consumption of 2.25 kg in 1961. India ranked 30th among 156 countries in per capita onion consumption, climbing 11 places over the past decade. In 2021, India led its neighboring countries in onion consumption, surpassing Bangladesh (15.6 kg), China (14.6 kg), Pakistan (7.72 kg), and trailing slightly behind Sri Lanka (17.4 kg per capita). Additionally, India exported over 1.71 million metric tons of fresh onions, valued at US$473.72 million, to major importing countries, including Bangladesh, Malaysia, Sri Lanka, the UAE, Nepal, Indonesia, Qatar, Vietnam, Kuwait, and Oman (APEDA, 2024). However, during years of unpredictable production shortfalls caused by unfavorable weather conditions, India has intermittently imported onions from several countries, including Middle Eastern nations, Egypt, Afghanistan, China, Iran, Pakistan, Turkey and Ukraine (Roy and Roy, 2022). To stabilize domestic prices and ensure market supply, the government maintains an annual onion buffer stock of 0.50 to 1.00 million tons under the price stabilization fund. This recurring supply-demand imbalance highlights the volatility and critical importance of onion production and trade in India. These challenges have prompted frequent government interventions, including export bans, imports and price controls, to stabilize the domestic market. The fluctuating gap between onion production and the demand-supply chain significantly impacts its market dynamics both nationally and globally, making it a key agricultural crop that garners substantial attention from breeders, policymakers and stakeholders (Premi and Premi, 2017; Kalamkar and Sharma, 2019).

Reasons behind fluctuations in onion production and demand-supply chain:

The fluctuations in onion area, production, harvest losses, and lower productivity in India are driven by multiple factors such as agronomic, climatic, breeding constrain in onion improvement and improper planning and market strategies. Beniwal et al. (2022) highlighted that seasonality significantly influences onion price trends, while Maity and Sharangi (2013) emphasized that proper planning of production, post-harvest management, and effective marketing strategies could help farmer's secure better prices for high-quality produce. However, breeders, scientists, and researchers attribute the primary causes of low production, market shortages, and price volatility to onion breeding constrains and weather variability induced by global warming. Frequent heat waves, high temperatures, droughts, and heavy rainfall events disrupt the onion supply chain, often triggering price spikes (Gedam et al. 2021; Godke et al. 2018; Mahajan and Gupta, 2015; Kavalgi et al. 2025; Economic Survey, 2024-25). Despite being a leading global onion producer, India's productivity (17.36 t/ha) remains significantly lower than that of other major onion-growing countries, such as the Republic of Korea (85.40 t/ha), the USA (56.03 t/ha), Spain (54.72 t/ha), and Australia (54.50 t/ha) (Anonymous, 2023). These countries benefit from a geographical advantage, being located between 30° and 50° North latitude, which allows the cultivation of long-day onion cultivars (>14 hours of photoperiod) with a longer growing season (>180 days), enhancing yields. Conversely, onion cultivation in India, occurring predominantly between 12° and 25° North latitude, is limited to short-day and intermediate-day varieties grown under subtropical to tropical conditions with an 11 to 11.5 hours photoperiod and shorter growth duration of 90-120 days, contributing to lower productivity. Additionally, the lower productivity is largely due to the widespread cultivation of open-pollinated varieties (OPVs) and locally produced seeds with minimal quality control, which have lower yield potential, improper crop management, plant protection measures and seasonal variations. In contrast, high-yielding countries predominantly cultivate F_1 hybrids, which exhibit superior heterosis and greater uniformity in horticultural and morphological traits, resulting in significantly higher productivity (Mahajan et al. 2021). Despite over 60 years of onion research in India, the absence of commercial hybrids from the public sector at the national level continues to hinder productivity gains (Kumar et al. 2015; Khar and Tomar, 2019). Furthermore, onion breeding faces several challenges due to its highly cross-pollinated nature, ranking second only to carrot in terms of inbreeding depression severity. Its two-year seed-to-seed life cycle makes the breeding process time-intensive. Being a photo-thermo-sensitive crop, onions are classified into short-day, intermediate-day, and long-day types based on their day-length requirements for bulb formation. However, changing climatic conditions increasingly impact onion production, further complicating cultivation. Moreover, onion is considered a genomically orphan crop with a large genome size (>16 Gb), making genomic research and breeding advancements challenging. For hybrid development, the creation of inbred lines is essential; however, this process is highly time-consuming, requiring approximately 10-12 years. The lack of proper genealogy records, limited research collaborations, and the absence of coordinated breeding programs further impede breeding progress. Despite the rising demand for onions, breeding activities in India remain significantly constrained by these complex genetic, physiological, and environmental factors (Khar and Singh, 2020; Mahajan and Gupta, 2023).

Needs and future breeding strategies:

The rising domestic consumption and increasing export demand for Indian onions underscore the urgent need to enhance production capacity to meet the growing requirements for domestic use, buffer stock, and exports. With India's expanding population, onion demand is projected to rise further, posing a significant challenge to food security. However, due to limited land availability and diminishing agricultural resources, the horizontal expansion of onion cultivation is no longer viable. Therefore, vertical expansion through advanced breeding strategies is essential, with a focus on developing high-yielding, nutritionally rich, and biotic and abiotic stress tolerant onion varieties and hybrids. Integrating modern breeding techniques into onion improvement programs is crucial for optimizing yield, enhancing quality, and maximizing economic returns (Godke *et al.,* 2018; Mahajan and Gupta, 2015; Khar and Singh, 2020). While F_1 hybrids generally outperform open-pollinated varieties (OPVs) globally in terms of yield and uniformity, OPVs remain economically viable in India due to their lower seed costs and suitability for saving of seed cost. Furthermore, OPVs offer greater genetic diversity and adaptability, making them more resilient under diverse agro-climatic conditions. In India, both high-yielding varieties (HYVs) and farmers' varieties (FVs) are widely cultivated across different seasons and agro-ecosystems. Brewster (2008) and Khokhar (2017) emphasized the critical role of genetically superior genotypes in enhancing onion bulb yield. Genetic improvement through plant breeding has significantly contributed to increased onion productivity, with bulb yields nearly doubling over time. To date, over 100 onion varieties, including eight multiplier onion varieties and a few F_1 hybrids, such as Arka Lalima and Arka Kirthiman (IIHR), POH-1 (PAU) and DOGR hybrids have been developed and released by various public sector organizations in India, catering to different bulb colours and growing seasons (Mahajan and Gupta, 2023). Onion varieties and hybrids released by different research institutes in India given in Table 1 and 2. The choice between F_1 hybrids and OPVs significantly influenced by several factors such as performance, economic viability, and technical factors (Singh and Agrawal, 2019). Therefore, a comparative assessment of the performance and economic returns of F_1 onion hybrids versus OPVs highlights the distinct advantages and challenges associated with each cultivation system.

Table 1: Onion varieties released through AICRP & AINRPOG and recommended for cultivation (Mahajan and Gupta, 2023).

S. No.	Name of Varieties	Special Feature	Yield potential (t/ha)	Institutes	Recommended zones & seasons	Year of Release
1	Punjab Selection	Red	20 t/ha	PAU, Ludhiana	IV, VII & VIII	1975
2	Pusa Red	Red	25 t/ha	ICAR-IARI, New Delhi	IV, VII & VIII	1975
3	Pusa Ratnar	Red	30-40 t/ha	ICAR-IARI, New Delhi	IV & VI	1975

S. No.	Name of Varieties	Special Feature	Yield potential (t/ha)	Institutes	Recommended zones & seasons	Year of Release
4	S-131	White	27-30 t/ha	ICAR-IARI, New Delhi	-	1977
5	N-257-9-1	White	25 t/ha	Agriculture Department, Maharashtra	LK & *Rabi*	1985
6	N-2-4-1	Red	30-35 t/ha	Agriculture Department, Maharashtra	LK & *Rabi*	1985
7	Line-102	Red	25-27 t/ha	ICAR-IARI, New Delhi	I, IV, VI & VII	1987
8	Arka Kalyan	Red	33.5 t/ha	ICAR-IIHR, Bengaluru	IV, VI, VII & VIII	1987
9	Arka Niketan	Red	34 t/ha	ICAR-IIHR, Bengaluru	IV, VII & VIII	1987
10	Agrifound Dark Red	Dark Red	30-40 t/ha	NHRDF, Nashik	IV	1987
11	VL-3	Red	20-25 t/ha	VPKAS, Almora	I	1990
12	Agrifound Light Red	Light Red	30-32.5 t/ha 30-35 t/ha	NHRDF, Nashik	VI & VIII	1993
13	Punjab Red Round	Red	30 t/ha	PAU, Ludhiana	IV	1993
15	L-28 (NHRDF Red)	Red	25-30 t/ha	NHRDF, Nashik	IV & VII	2006
16	HOS-1	Red	20-25 t/ha	HAU, Hissar	VI	2006
17	Bhima Raj	Red	24-26 t/ha (*Kharif*) & 40-45 t/ha (LK) & 25-30 t/ha (*Rabi*)	ICAR-DOGR, Pune	VI	2008
18	Bhima Red	Red	19-21 t/ha (*Kharif*) & 45-50 t/ha (LK) & 30-32 t/ha (*Rabi*)	ICAR-DOGR, Pune	VII for *rabi* & II, V & VI** for *kharif*	2009 & 2013
19	PKV White (Akola Safed)	White	30-35 t/ha	PDKV, Akola	VI	2009

S. No.	Name of Varieties	Special Feature	Yield potential (t/ha)	Institutes	Recommended zones & seasons	Year of Release
20	Phule Samarth (RHOR-S1)	Red	30-31 t/ha	MPKV, Rahuri	VI & VIII	2009
21	Bhima Kiran	Red	28-32 t/ha (*Rabi*)	ICAR-DOGR, Pune	III & VI*	2010
22	Line-355 (NHRDF Red-2)	Red	35-37.5 t/ha	NHRDF, Nashik	III, IV & VI*	2010
23	Bhima Shakti	Medium Red	35-40 t/ha (LK) & 28-30 t/ha (Rabi)	ICAR-DOGR, Pune	III, IV, V & VI*	2011
24	Bhima Shweta	White	18-20 t/ha (*Kharif*) & 26-30 t/ha (*Rabi*)	ICAR-DOGR, Pune	III, V & VI* for *rabi* & IV, V & VI** for *kharif*	2011 & 2013
25	Sel-126	Brown	25 t/ha	ICAR-IARI, New Delhi	III, IV & V*	2011
26	Bhima Super	Pinkish	20-22 t/ha (*Kharif*) & 40-45 t/ha (LK)	ICAR-DOGR, Pune	II, IV, V & VI** for *kharif*	2013
27	Bhima Dark Red	Dark Red	22-24 t/ha (*Kharif*)	ICAR-DOGR, Pune	II, IV, V & VI**	2013
28	Bhima Shubhra	White	18-20 t/ha (*Kharif*) & 36-42 t/ha (LK)	ICAR-DOGR, Pune	IV, V & VI** for *kharif*	2013
29	Bhima Safed	White	18-20 t/ha (*Kharif*)	ICAR-DOGR, Pune	IV, V & VI** for *kharif*	2015
30	Bhima Light Red	Light Red	36-40 t/ha (*Rabi*)	ICAR-DOGR, Pune	VI	2016
31	Sel. 153-1	Creamis h Yellow	33-35 t/ha	ICAR-IARI, New Delhi	II	2016
32	Col. 819	Red	-	NHRDF, Nashik	II	2016

Note: Zone I = Himachal Pradesh and U.P. Hills, (**Srinagar, Almora, Mukteshwar, Palampur, Ooty); Zone II = West Bengal and Assam, (**Jammu, Ludhiana, Delhi, Karnal, Hissar, Durgapura); Zone III= Sikkim, Meghalaya, Manipur, Nagaland, Mizoram, Tripura, Arunachal Pradesh and Andaman and Nicobar Islands, (*Delhi, UP, Haryana, Bihar and Punjab); Zone IV = Punjab, Tarai region of U.P. and Bihar, (*Rajasthan and Gujarat), (**Jabalpur, Raipur, Chiplima, Akola, Jhalawar); Zone V = Chhattisgarh, Orissa and Andhra Pradesh, (*MP, Chhattisgarh and Orissa), (**Junagadh, Nashik, Rahuri, Pune); Zone VI = Rajasthan, Gujarat, Haryana and Delhi, (*Maharashtra, Karnataka and Andhra Pradesh), (**Bagalkot, Bangalore, Coimbatore, Dharwad); Zone VII = Madhya Pradesh and Maharashtra, Zone VIII = Karnataka, Tamil Nadu and Kerala.

Table 2: Onion varieties released by different research institutes in India (Kumar et al.2015; Mahajan and Gupta, 2023).

S. No.	Variety/ Hybrids	Special feature	Yield potential (t/ha)	Recommended season	Organization	Year of release
1	N-53	Red	25 t/ha	*kharif*	Agriculture Department, Maharashtra	1975
2	N-2-4-1	Red	30-35 t/ha	*rabi* and LK		1985
3	N-257-9-1	White	25 t/ha	*rabi*		1985
4	Baswant-780	Red	25 t/ha	*kharif*	MPKV, Rahuri	1989
5	Phule Safed	White	25-30 t/ha	LK and *rabi*		1994
6	Phule Swarna	Yellow	24 t/ha	*rabi* and LK		2001
7	Phule Samarth	Red	30 t/ha	LK		2006
8	Early Grano (Long Day)	Yellow	50-60 t/ha	*rabi*	ICAR-IARI, Katrain	1975
9	Brown Spanish (Long Day)	Brown	28-30 t/ha	Hills		1975
10	Pusa White Round	White	30-32.5 t/ha	*rabi*	ICAR-IARI, New Delhi	1975
11	Pusa White Flat	White	32.5-35 t/ha	*rabi*		1975
12	Pusa Red	Red	25 t/ha	*rabi*		1975
13	Pusa Ratnar	Red	30-40 t/ha	*rabi*		1975
14	Pusa Madhavi	Red	30 t/ha	*rabi*		1987
15	Pusa Shobha	Brown	25 t/ha	*rabi*		2012
16	Pusa Riddhi	Dark red	32 t/ha	*rabi*		2013
17	Pusa Sona	Yellow	33-35 t/ha	*rabi*		2017
18	Arka Pragati	Red	20 t/ha	*kharif* and *rabi*	IIHR, Bengaluru	1984
19	Arka Niketan	Red	34 t/ha	*rabi* and LK		1987
20	Arka Kalyan	Red	33.5 t/ha	*kharif*		1987
21	Arka Pitamber	Yellow		*rabi*		2006
22	Arka Bindu	Red	25 t/ha	*kharif*, LK and *rabi*		2006
23	Arka Ujjwal (Multiplier onion)	Red	20-25 t/ha	*rabi*		2010
24	Arka Swadista	White	30 t/ha	*rabi*		2010

S. No.	Variety/ Hybrids	Special feature	Yield potential (t/ha)	Recommended season	Organization	Year of release
25	Arka Vishwas (Rose onion)	Dark red	30 t/ha	*kharif* and *rabi*		2011
26	Arka Sona	Yellow	45 t/ha	*rabi*		2011
27	Arka Bheem (Syn-6) (tri parental synthetic)	Red	47 t/ha	*rabi*		2011
28	Arka Akshay (Syn-4) (tri-parental synthetic)	Dark Red	45 t/ha	*rabi*		2011
29	Arka Lalima (F_1 hybrid)	Red	47 t/ha	*kharif* and *rabi*		1998
30	Arka Kirthiman (F_1 hybrid)	Red	47 t/ha	*kharif* and *rabi*		1998
31	Arka Yojith	White	25-30 t/ha	*rabi*		-
32	Hissar-2	Red	33.5 t/ha	*rabi*	HAU, Hissar	1976
33	HOS-1	Red	20-25 t/ha	*rabi*		2006
34	Hissar Onion-3	Bronze	32-35 t/ha	*rabi*		2010
35	Hissar Onion-4	Rose Red	32.5 t/ha	*rabi*		2016
36	Agrifound Light Red	Red	30-35 t/ha	*rabi* and LK	NHRDF, Nashik	1988
37	Agrifound Dark Red	Red	30-40 t/ha	*kharif*		1996
38	NHRDF Red (L-28)	Red	25-30 t/ha	*rabi*		2006
39	NHRDF Red-2 (L-355)	Red	35-37.5 t/ha	*rabi*		2012
40	NHRDF Red -3 (L-625)	Light bronze	35-40 t/ha	*rabi*		-
41	NHRDF Red-4 (L-744)	Dark red	35-40 t/ha	*rabi*		2016
42	NHRDF Fursungi (L-719)	Red	38-40 t/ha	*rabi*		2017
43	Agrifound Rose	Red	25-30 t/ha	*Rabi*		1987

S. No.	Variety/ Hybrids	Special feature	Yield potential (t/ha)	Recommended season	Organization	Year of release
44	Agrifound Red (Multiplier)	Red	18-20 t/ha	*kharif* and *rabi*		1987
45	Advance Line-863	Dark red	28-30 t/ha	*kharif* and *LK*		-
46	Advance Line-883	Dark red	30-32.5 t/ha	*kharif and early kharif*		-
47	Advance Line-857	White	35-40 t/ha	*rabi*		-
48	Advance Line-849	Red	37.5-42.5 t/ha	*rabi*		
49	Agrifound White	White	20-25 t/ha	*rabi*		1994
50	VL-67 (Long Day)	Red	20-25 t/ha	Hills	VPKAS, Almora	1973
51	VL-3 (Long Day)	Red	20-25 t/ha	Hills		1990
52	Udaipur 101	Red	20-30 t/ha	*rabi*	RAU, Rajasthan	
53	Udaipur 102	White	30-35 t/ha	*rabi*		
54	Udaipur 103	Red	20-30 t/ha	*rabi*		
55	PKV White (Akola Safed)	White	30-35 t/ha	*rabi*	PDKV, Akola	2009
56	Gujarat White Onion (GWO)-1	White	30-35 t/ha	*rabi*	GAU, Junagadh	2000
57	Gujarat Junagadh Red Onion- 11 (GJRO-11)	Red	32.35 t/ha	*rabi*		2016
58	Gujarat Junagadh White Onion- 3 (GJWO-3)	White	23.15-40.84 t/ha	*rabi*		2016
59	Kalyanpur Red Round	Red	20-25 t/ha	*rabi*	CSAUAT, Kanpur	1983
60	Punjab Selection	Red	20 t/ha	*rabi*	PAU, Ludhiana, Punjab	1973
61	Punjab Red Round	Red	30 t/ha	*rabi*		1993
62	Punjab-48 (S-48)	White	30 t/ha	*rabi*		1978
63	Punjab White	White	31-33 t/ha	*rabi*		1998
64	Punjab Naroya (PBR-5)	Red	37-40 t/ha	*rabi*		1997

S. No.	Variety/ Hybrids	Special feature	Yield potential (t/ha)	Recommended season	Organization	Year of release
65	Punjab Onion Hybrid-1 (POH-1)	Light Red	22.10 t/ha	*rabi*		2022-23
66	CO-1 (Multiplier onion)	Red	10 t/ha	*kharif* and *rabi*	TNAU, Coimbatore	1965
67	CO-2 (Multiplier onion)	Red	12 t/ha	*kharif* and *rabi*		1978
68	CO-3 (Multiplier onion)	Red	16 t/ha	*kharif* and *rabi*		1982
69	CO-4 (Multiplier onion)	Red	18 t/ha	*kharif* and *rabi*		1984
70	CO-5 (Multiplier onion)	Pink	18 t/ha	*kharif* and *rabi*		2001
71	MDU-1	Red	15 t/ha	*rabi*		1982
72	CO-6	Pink	18.90 t/ha	*kharif* and *rabi*		2020
73	Rajasthan Onion-1 (RO-1)	Red	32-36 t/ha	*rabi*	RARS, Durgapura	2004
74	Arpita (RO-59)	Red	35-40 t/ha	*rabi*		2005
75	RO-252	Red	29-30 t/ha	*rabi*		2011
76	Bhima Raj	Dark Red	24-26 t/ha (*Kharif*) & 40-45 t/ha (LK) & 25-30 t/ha (*Rabi*)	*kharif* and *rabi*	DOGR, Pune	2008
77	Bhima Red	Medium Red	19-21 t/ha (*Kharif*) & 45-50 t/ha (LK) & 30-32 t/ha (*Rabi*)	*kharif* and *LK*		2009 & 2013
78	Bhima Kiran	Light Red	28-32 t/ha (*Rabi*)	*rabi*		2010
79	Bhima Shakti	Medium Red	35-40 t/ha (LK) & 28-30 t/ha (*Rabi*)	*LK* and *rabi*		2011

S. No.	Variety/ Hybrids	Special feature	Yield potential (t/ha)	Recommended season	Organization	Year of release
80	Bhima Shweta	White	18-20 t/ha (*Kharif*) & 26-30 t/ha (*Rabi*)	*kharif* and *rabi*		2011 & 2023
81	Bhima Super	Pink	20-22 t/ha (*Kharif*) & 40-45 t/ha (LK)	*kharif, LK* and *rabi*		2013
82	Bhima Shubhra	White	18-20 t/ha (*Kharif*) &36-42 t/ha (LK)	*kharif* and *LK*		2013
83	Bhima Dark Red	Dark Red	22-24 t/ha	*kharif*		2013
84	Bhima Safed	White	18-20 t/ha	*kharif*		2015
85	Bhima Light Red	Light Red	36-40 t/ha	*rabi*		2016

Factors contributing to the continued dominance and preference for OPVs over F_1 hybrids in onion cultivation in India:

The continued dominance of open-pollinated onion varieties (OPVs) in India is attributed to several key factors, making them the preferred choice among farmers and the predominant cultivars in onion production. The slow progress of hybrid breeding programs in this vital cash crop has resulted in farmers' heavy reliance on OPVs (Singh and Khar, 2023). These varieties exhibit broader genetic diversity and have undergone decades of local adaptation, enhancing their resilience to diverse agro-climatic conditions and environmental stresses (Cramer, 2000). Moreover, India primarily cultivates short-day onions with a growing period of 90–120 days, ideally suited to the tropical and subtropical climate (12°–25° North latitude) (Kumar et al. 2015). OPVs are also more economical, offering lower seed costs (onion seed rate: 7-10 kg/ha) and the advantage of seed saving for subsequent planting seasons, significantly reducing input expenses. Their adaptability to low-input farming systems makes them particularly suitable for small and marginal farmers, who constitute the majority of onion growers in India (FAO, 2014). Consequently, Indian farmers continue to favor OPVs for their proven performance, stability, and cost-effectiveness, reinforcing their dominance over F_1 hybrids (Singh and Khar, 2023; Gupta and Singh, 2016). Several studies have demonstrated that locally adapted OPVs often perform as well as or even better than hybrids in terms of bulb traits, yield, and resistance to biotic and abiotic stresses, particularly in specific agro-ecological regions (Dangi et al. 2018; Khade et al. 2020). For instance, Cramer (2000) conducted a comparative study in Mexico, evaluating 18 hybrid and 23 OP varieties with short to intermediate-day bulbing responses

during the fall season. While hybrids exhibited superior morphological traits (plant height and leaf number), they did not outperform OPVs in yield, yield-related traits, or disease resistance. Most OPVs performed equally well or better due to their local adaptation, unlike hybrids bred for different agro-climatic conditions. Additionally, OPVs maintained genetic variation through cross-pollination, enhancing their resilience and stability under local conditions, whereas hybrids suffered from inbreeding depression. Similarly, Dangi et al. (2018) characterized 58 onion genotypes, including landraces, OPVs, hybrids, and breeding materials, based on morphological and biochemical traits, revealing substantial genetic variability. Interestingly, OPVs displayed superior performance for most traits, with Pusa White Flat (PWF) recording the highest yield, 106BS2 showing the maximum total soluble solids (TSS), Arka Kalyan exhibiting the highest dry matter content, Red Creole-3 recording the maximum pyruvic acid content, and Juni displaying the highest total phenolic content. The superior performance of OPVs over hybrids was attributed to the exotic nature of hybrids, which required further adaptation to local conditions. Likewise, Khade et al. (2020) evaluated heterosis for yield and related traits in 12 CMS-based short-day onion hybrids against the commercial hybrid 'Flare,' revealing significant trait variability. However, none of the hybrids consistently outperformed OPVs across all morphological, yield, and quality traits. The authors emphasized the need for multi-locational testing to accurately estimate gene action governing yield and related traits, ensuring the identification of promising hybrids for commercial cultivation. Additionally, several studies have demonstrated that locally adapted open-pollinated onion varieties (OPVs) often perform as well as or even better than hybrids in terms of bulb traits, yield, and resistance to biotic and abiotic stresses, particularly in specific agro-ecological regions. Sharma et al. (2014) evaluated onion varieties under the low hill conditions of Himachal Pradesh, highlighting their adaptability and yield potential. Islam et al. (2019) conducted a study on variability, heritability, and trait associations for bulb and antioxidant traits in onion varieties, revealing substantial genetic diversity and stability. Manjunathagowda et al. (2019) assessed the performance of OPV onion genotypes under the southern dry zone of Karnataka, reporting promising results in terms of yield and stress tolerance. Similarly, Gupta et al. (2020) evaluated *Kharif* onion varieties in the Malwa agro climatic zone of Madhya Pradesh, identifying superior-performing genotypes. In a subsequent study, Manjunathagowda et al. (2021) analyzed variability and genetic diversity among selfed lines (S1) of onion, emphasizing their potential for genetic improvement. Recently, Gupta et al. (2024) assessed the genetic diversity and stability performance of 38 onion genotypes, highlighting their significant plant growth and bulb production ability. Collectively, these studies underscore the superior adaptability, stability, and productivity of region-specific OPVs, reaffirming their suitability for cultivation under diverse agro-ecological conditions.

Reasons for the limited popularity and commercialization of onion F_1 hybrids in India, despite their potential advantages:

The limited progress and commercialization of heterosis breeding and F_1 hybridization programs in onion cultivation in India are attributed to several key factors. Firstly, onion is a biennial, cross-pollinated and heterozygous diploid crop with a prolonged breeding cycle, requiring 10-12 years to

develop and stabilize inbred lines through conventional methods. The small size of onion florets makes hand emasculation and pollination labor-intensive and impractical for large scale hybrid seed production (Dhatt, 2023). Although the discovery of cytoplasmic male sterility (CMS) has facilitated hybrid development, its commercial success is largely limited to long-day onion-producing countries such as North America, Europe, and Japan (Singh and Khar, 2023). In India, hybridization efforts are hindered by the lack of stable male sterile (A-line) and maintainer (B-line) lines. Despite initiatives by public institutions like PAU Ludhiana, IIHR Bengaluru, DOGR Pune, and IARI Delhi to develop CMS-based tropical onion hybrids, progress has been slow due to the predominance of open-pollinated varieties (OPVs) and the laborious nature of parental line maintenance (Dhatt, 2023). Furthermore, existing CMS lines, such as Pusa Red and *Allium galanthum*, often suffer from sterility breakdown under unfavorable conditions, including high temperatures and environmental stress, reducing their reliability (Khar and Singh, 2020). The labor-intensive and costly maintenance of male sterile lines, requiring skilled labor and significant financial resources, renders large-scale hybrid seed production economically unviable. Additionally, the limited expression of substantial heterosis for desirable bulb traits, such as yield, shape, colour, quality, and storage characteristics, further restricts hybrid adoption. Even when heterosis results in larger bulbs, it does not align with Indian consumer preferences and export requirements, which favor medium-sized, dark red-coloured bulbs (NHRDF, 2023). Moreover, F_1 hybrid cultivation demands a higher seed cost and a 100% seed replacement ratio, making it 5-8 times more expensive than OPV seeds, thereby limiting its affordability and adoption among small and marginal farmers (Mohan et al. 2021). Large-scale F_1 hybrid seed production is further constrained by the crop's short seed viability (<1 year), making long-term storage and multi-season usage difficult (Yalamalle *et al.,* 2020). Additionally, most available F_1 hybrids in India are relatively new and lack extensive multi-locational trials or large-scale performance assessments, limiting their credibility and acceptance among farmers (Singh and Khar, 2021). Their exotic origin makes them less adapted to local agro-climatic conditions, resulting in inconsistent performance across different regions. Consequently, the slow progress of hybrid breeding programs, combined with the proven adaptability, stability, and cost-effectiveness of OPVs, continues to restrict the commercialization of F_1 hybrids in India (Dangi et al. 2018; Khade et al. 2020). However, several studies have demonstrated the potential of F_1 onion hybrids for superior bulb yield, stress tolerance, and quality traits in specific agro-ecological regions. Khade et al (2020) evaluated 12 CMS-based short-day hybrids, identifying PR-121 × EG as the most promising, exhibiting 81.6% heterobeltiosis and 79.7% standard heterosis for bulb yield. Similarly, Khade et al. (2012) assessed 12 CMS-based hybrids and reported A × EG as superior for bulb yield, dimensions, and plant vigor, while A × RO-597 excelled in phytochemical and mineral content. Ahmad et al. (2021) identified MKS-SGB × MKS-GBP-01 as the most heterotic combination, with 55.77% commercial heterosis for bulb yield. Gupta et al. (2018) evaluated 110 F_1 red onion hybrids using five male sterile lines and 22 elite pollinators, identifying DOGR Hy-156, DOGR Hy-173, and DOGR Hy-179 as the most promising for early maturity, high yield, and storability, with marketable yields up to 43.12 t/ha. Ajjappalavara *et al.* (2022) evaluated 31 coloured onion hybrids, with RH-13 recording the highest bulb diameter and weight, RH-3 and WH-5 showing the highest TSS content, and RH-9 exhibiting the least thrips infestation. Narina et al. (2020) investigated the post-harvest storage quality of 60 CGMS-based

onion hybrids, identifying 54 hybrids with superior bulb yield, size, and storability over five months. The hybrid 75 msms × New Collection from Daultabad-1 x P2 exhibited 85.8% heterosis over the better parent and 113.73% over the standard check, while (102-1 × 106) × Red Creole-1 x P2 demonstrated early maturity (113 days). These studies underscore the potential of F_1 hybrids for high-yielding, stress-tolerant, and superior-quality onion production, making them ideal candidates for commercial cultivation. Additionally, Kiani et al. (2023) highlighted the superior antioxidant status of cultivars like Azarshahr, Kurdistan, and Esfahan over short-day hybrids and local landraces, making them promising sources of valuable phytochemicals for breeding programs aimed at enhancing onion quality. The study further emphasized the importance of preserving and enhancing the productivity of local landraces as sustainable alternatives to exotic F_1 hybrids, promoting long-term sustainability in onion cultivation.

Importance of heterosis breeding or F_1 hybridization program in onion crop:

Heterosis breeding holds immense potential for enhancing onion productivity, earliness, uniformity in bulb maturity, quality and storage life, while improving economically significant traits such as bulb colour, shape, size, and yield-contributing characteristics. Consequently, hybrid onion cultivation has expanded considerably in developed countries, and both the public and private sectors in India are increasingly prioritizing F_1 hybrid development to capitalize on hybrid vigor. Onion, being a cross-pollinated crop, exhibits substantial variability in maturity, bulb traits, day-length requirements for bulbing, vernalization for flowering, total soluble solids (TSS), and dry matter content (Singh et al. 1998). Among the key qualitative traits, male sterility plays a pivotal role in hybrid breeding, as male-sterile plants fail to produce viable pollen, facilitating exclusive cross-pollination. This characteristic is effectively exploited for commercial F_1 hybrid seed production, which demonstrates superior hybrid vigor for various economically important traits. However, in the absence of male sterility, controlled cross-pollination can only be achieved through labor-intensive and commercially unviable hand emasculation and pollination techniques, limiting its application to experimental breeding (Gupta and Singh, 2016). The discovery of cytoplasmic-genic male sterility (CGMS) by Jones and Clarke (1947) revolutionized onion heterosis breeding, enabling large-scale hybrid seed production globally in both long-day and short-day onion types. Their pioneering work, based on the male-sterile genetic stock of the 'Italian Red' cultivar, introduced a reliable CMS system, forming the foundation for hybrid onion breeding programs (Jones and Clarke, 1947). In India, CGMS-based hybrid breeding gained momentum in the 1980s at IIHR (Bangalore), IARI (New Delhi), and MPKV (Rahuri). At IIHR, the first male sterility instance was reported in the local cultivar 'Nasik White Globe' (Pathak et al., 1980), while at IARI, it was identified in the commercial variety 'Pusa Red.' However, only two hybrids, Arka Kirthiman and Arka Lalima, were released by IIHR after developing CGMS lines with their respective maintainers. Subsequent studies revealed that tapetal abnormalities and histochemical changes, rather than meiotic abnormalities, were primarily responsible for male sterility in onion (Saraswathi and Veere Gowda, 2006; Tomar et al. 2019). Despite the emphasis on hybrid breeding for enhanced productivity and stress tolerance since the 1960s, India still lags behind countries like the USA, Japan,

and Korea in hybrid adoption. Globally, F_1 hybrids are increasingly preferred due to their higher yield potential, uniform bulb traits, and consistent performance. The commercial success of onion hybrids depends on the availability of stable inbred lines and a reliable male sterility mechanism. To develop widely adopted commercial hybrids in India, it is crucial to identify stable CMS lines from locally adapted populations using advanced molecular techniques. The application of molecular markers to identify and differentiate sterile, maintainer and pollen parent is essential for efficient heterosis breeding. Several studies have demonstrated the potential of molecular markers linked to the Ms locus and male-sterile cytoplasms (CMS-S and CMS-T) for improving F_1 hybrid development, particularly in terms of bulb yield, stress tolerance, and quality traits. Researchers have successfully developed molecular markers associated with male-sterile cytoplasms (CMS-S and CMS-T), male-fertile normal (N) cytoplasm, and nuclear male-fertility restorers (*Ms* locus), significantly enhancing the efficiency of hybrid breeding through marker-assisted selection (MAS). These markers facilitate the precise identification and isolation of male-sterile lines, their maintainer lines, and restorer of fertility lines, essential for large-scale hybrid seed production. Manjunathagowda (2021) highlighted the practical utility of molecular markers in accurately distinguishing cytoplasmic types and identifying the *Ms locus,* accelerating the development of F_1 hybrids and enhancing hybrid seed purity. Similarly, Sharma et al. (2023) emphasized the effectiveness of CGMS-based hybrid breeding, which involves a three-line system comprising the male-sterile line (A), its isogenic fertile maintainer line (B), and the fertility restorer line (R). Their study demonstrated that molecular marker-assisted methods are more efficient than conventional breeding techniques in identifying S, T, and N cytoplasms, as well as maintainer and restorer lines, enabling the development of high-heterosis onion hybrids. Bal (2023) reviewed the genetic and molecular aspects of male sterility in onion, underscoring the significance of molecular markers in streamlining hybrid breeding programs. Abbasi (2023) further highlighted the effectiveness of CMS hybrid seed production systems, facilitated by molecular markers linked to male-sterile cytoplasms and the Ms locus, in enhancing hybrid seed purity and productivity. Collectively, these studies underscore the transformative potential of molecular markers in accelerating onion hybrid breeding, improving hybrid seed quality, and boosting productivity through the efficient exploitation of heterosis.

Conclusion

F_1 hybrids in onion offer significantly higher yield potential (20-50% more than OPVs) due to hybrid vigor, with enhanced uniformity in bulb size, shape and maturity. They also exhibit superior storability, greater resistance to biotic and abiotic stresses and are more suitable for mechanical harvesting. However, hybrid seed production requires controlled conditions, skilled personnel and substantial resources, making it costlier. Despite the higher initial investment, F_1 hybrids provide greater economic returns due to their higher yield and quality, making them preferable for commercial farming. In contrast, OPVs, although offering moderate yield potential and less uniformity, remain economically viable for small-scale and subsistence farmers due to their lower seed costs and adaptability. OPV seed production is simpler, less resource-intensive and allows for seed saving, reducing input expenses. However, OPVs face challenges such as inconsistent bulb quality, higher susceptibility to bolting, and lower storability, making them less competitive in

commercial markets. In India, the dominance of OPVs is primarily driven by the lack of stable male sterile lines, limited hybrid research, and the higher production costs associated with hybrids. To promote hybrid onion cultivation, it is essential to strengthen breeding programs by developing stable male sterile lines from locally adapted populations, identifying heterotic groups through multi-location testing, and integrating advanced biotechnological approaches. Mechanization and collaborative initiatives between the public and private sectors are necessary to reduce production costs and meet the rising demand for fresh, processing and exotic onion markets. Simultaneously, improving OPVs for higher yield, uniformity and stress tolerance is equally important to support resource-limited farmers and preserve genetic diversity. By balancing hybrid and OPV development through targeted research, enhanced seed systems, and collaborative efforts, India can significantly boost onion productivity, quality and market competitiveness, ultimately benefiting both commercial and small-scale farmers.

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Proceedings of the Brainstorming Session on Hybrid Breeding in Onion Organized by ICAR-DOGR at NASC Complex, New Delhi

ICAR-DOGR, Pune and Horticultural Science Division, ICAR, New Delhi organized the Brainstorming Session on "Hybrid Breeding in Onion: Innovations, Challenges and Future Prospects" in collaboration with NHRDF, New Delhi on 16.4.2025 at NASC Complex, New Delhi. The aim of this session was to strengthen the hybrid breeding program of onion in mission mode and to streamline the hybrid evaluation program under AINRPOG. Around 22 experts from ICAR institutes, SAU's, Private Sector etc., attended the program. Dr. Sudhakar Pandey, ADG (FVS & MP) (HS) ICAR, was the chief guest and chaired the session, Dr. V Mahajan, Director, ICAR-DOGR & Dr. AS Dhatt, Director Research, PAU were the Co-Chairmans and Dr. Amar Jeet Gupta, Pr. Scientist (Veg. Sci.) was the Organizing Secretary.

Program started with welcome address by Dr. V Mahajan and he highlighted the status of onion hybrids in India. Dr. Sudhakar Pandey explained the importance of hybrid varieties in onion and the need for onion hybrids in India. He emphasized on the development of stable hybrid varieties by collaborative research, which are better than OPVs to increase the productivity of onion. Dr. AS Dhatt expressed his views and gave information about hybrid breeding at PAU. He also emphasized on development of true inbreds through doubled haploids along with development of male sterile lines in locally adapted varieties suited to Indian conditions and use of molecular markers to characterize these lines.

The meeting started with a technical session consisting of presentations by scientists from ICAR-DOGR, IARI, IIHR, VPKAS, PAU and CITH regarding the current status and prospects of hybrid breeding in onion and way forward. Dr. Anshul Kumar, representative from private sector also shared his feedback regarding present position of hybrids in Indian market and the improvement required.

It was followed by panel discussion among experts which was moderated by Dr. PC Tripathi, Pr. Scientist (Hort), ICAR. In-depth discussions were held in this session including development of high yielding hybrid and recommendations were made.

The panel critically reviewed the existing breeding strategies and recent technological advancements. After a series of comprehensive technical presentations and in-depth discussions involving experts from various ICAR institutes, several key recommendations were made as follows.

Recommendations

1. To strengthen the onion hybrid breeding program, a strategic collaboration should be established among ICAR-DOGR, IARI, IIHR, VPKAS and PAU. This joint initiative should focus on

developing superior onion hybrids with desirable traits. A comprehensive mega project proposal, led by ICAR-DOGR, should be formulated and submitted to the ICAR Corpus Fund and NASF for funding support.

2. There is need to focus on development of large number of inbred lines along with stable and well-characterized male sterile (A) and maintainer (B) lines.
3. Hybrid development should be strengthened by making a greater number of crosses among heterotic combinations using cytoplasmic male sterility; systematically analyze general and specific combining abilities; and ensure hybrids exhibit stable performance with uniform bulb shape, size and desirable colour.
4. Develop male sterile lines in diverse background to create more heterotic and stable hybrid combination to enhance bulb quality as well as productivity.
5. Prioritize inter-specific hybridization to develop novel parental lines with enhanced traits for hybrid development.
6. Attention should be given to strengthening the development of trait specific inbred lines in onion.
7. Characterize available CMS lines using both physical and molecular markers to confirm their sterility and utility in hybrid breeding.
8. There is a need to create speed breeding technologies to accelerate the development of inbred lines and doubled haploids.
9. Rigorous screening of available parental lines should be done against major diseases like *Stemphylium* blight, anthracnose and purple blotch to identify tolerant and susceptible lines and utilization in hybrid development.
10. Use heterosis breeding to develop hybrids with better storability, aiming to limit post-harvest storage losses to below 15%.
11. Exchange need-based parental lines among centers to enhance breeding outcomes within timeline.
12. Breed for thermo-insensitive/ climate resilient hybrids to ensure wider adaptation and extended cropping seasons.
13. Tissue culture technique may be explored to large-scale multiplication of trait-specific inbred lines as well as male sterile and maintainer lines to accelerate the hybrid development program.
14. Developed heterotic cross combinations should be evaluated across multiple locations under AINRPOG to identify region-specific and high-performing hybrids.

Suggestions

1. ICAR should create a centralized system/platform for collaborative hybrid breeding to unify the efforts of breeders, researchers and other stakeholders.
2. Effective onion hybrid development requires team work including breeders, biotechnologists, cytologists, pathologists etc.

3. Employ various approaches including use of honey bees or alternative pollinators to boost up hybrid seed production.
4. Funding from the ICAR Corpus Fund should be secured to establish essential infrastructure, including dedicated net house facilities for maintaining true-to-type inbred and parental lines, as well as for upgrading critical seed production facilities to support hybrid development.
5. Secondly, financial support should be sought from the National Agricultural Science Fund (NASF) to strengthen basic research activities, particularly focusing on the development of male sterile lines and doubled haploid lines for enhancing the efficiency of onion hybrid breeding.







Photographs during the brainstorming session

Photographs during the brainstorming session

Photographs during the brainstorming session

Programme Schedule

Horticultural Science Division, ICAR, New Delhi
&
ICAR-DOGR, Pune & NHRDF, New Delhi

Brain Storming Session

"Hybrid Breeding in Onion: Innovations, Challenges and Future Prospects"

Chairman: Dr. S. K. Singh, DDG (Hort. Sci.)

Co-Chairman: Dr. Sudhakar Pandey, ADG; Dr. V. Mahajan, Director; Dr. A. S. Dhatt, DR, PAU

Organizers: Dr. V. Mahajan, Dr. P. K. Gupta & Dr. Amar Jeet Gupta, Organizing Secretary

Rapporteurs: Dr. Amar Jeet Gupta & Dr. Sabina Islam

16.04.2025
9.30 a.m.

NASC, Basmati Hall
II Floor, NASC, New Delhi

9.30 am	Registration	
10.00 am	Welcome	Dr. Vijay Mahajan
10.05 am	Opening Remark by Chairman	Dr. S. K. Singh
10.10 am	Status of Onion Hybrid Breeding in India and at PAU and future road map	Dr. J. S. Khosa
10.30 am	Onion Hybrid Breeding: An ICAR-DOGR Perspective and future road map	Dr. Amar Jeet Gupta
10.50 am	Status of Onion Hybrid Breeding at ICAR-IIHR and future road map	Dr. B. R. Raghu
11.10 am	***Tea Break***	
11.30 am	Status of Onion Hybrid Breeding at ICAR-VPKAS and future road map	Dr. N. K. Hedau
11.50 am	Status of Onion Hybrid Breeding at IARI and future road map	Dr. Sabina Islam
12.10 pm	Status of Onion Hybrid Breeding at ICAR-CITH and future road map	Dr. Geetika Malik
12.30 pm	Application of biotechnological tools in accelerating inbred line development	Dr. S. Anandhan
	Panel discussion: (5 mins each) **Moderator:** Dr. P. C. Tripathi, Principal Scientist, ICAR	
12.50 pm	Discussion and Planning of Program for Development of Parental Lines and Hybrids in Onion	Dr. A. S. Dhatt Dr. V. Mahajan Dr. B. S. Tomar Dr. P. K. Gupta Dr. Anshul Kumar
01.15 pm	Open Discussion	All Participants
01.45 pm	Special Remarks	Dr. Sudhakar Pandey, ADG (FVS & MP) (HS)
01.50 pm	Concluding Remarks	Dr. S. K. Singh, DDG (HS)
01.55 pm	Vote of Thanks	Dr. A. J. Gupta, Org. Sec.
2.00-3.00 pm	***Lunch***	
3.00-4.00 pm	Finalization of Proceedings	All participants

Dr. Sudhakar Panday (ADG) chaired the session in absence of DDG (Hort. Sci.)

भाकृअनुप
ICAR

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