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Perspectives of onion hybrid breeding in India: An overview

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ABSTRACT

Onion (*Allium cepa* L.), a major member of monocot family *Amaryllidaceae*, is an important bulbous vegetable crop used in almost every Indian kitchen. Being cultivated since antiquity, farmers rely on growing open pollinated varieties since hybrid development in this crop has not gained pace. Among important vegetables, F_1 hybrid development remains underutilized in onion especially in India and other developing countries as compared to other onion growing countries. Development of commercial hybrids in onion principally depends on the availability of suitable inbred lines against the backdrop of stable male sterile system. The future scope of commercial onion hybrids needs to be focused comprehensively for the identification of male sterile lines from Indian onion population by utilizing modern biotechnological tools. Molecular markers distinguishing cytoplasm and linked to restorer of male sterility, *Ms* locus, are important. Release of commercial hybrids from public sector would play a great role for breaking yield barriers and significant enhancement of productivity of onion under changing climate scenario and increasing domestic demand.

Keywords: *Allium cepa*, CMS, Cytoplasm types, F_1 hybrid onion, Heterosis, *Ms* locus

Onion (*Allium cepa* L., 2n=2x=16), belonging to the family *Amaryllidaceae,* possesses various health promoting properties like lipid-lowering, anti-diabetic, anti-hypertensive, anti-microbial, immune-protective and anti-obesity properties (Galavi *et al.* 2020). Since antiquity, Indian farmers cultivated onions for culinary purpose to augment flavour and for raw consumption. Indian short-day onion is preferred immensely because of their unique spiciness and taste properties in many countries for export (Khar and Saini 2016). Demand of onion in India is increasing day by day owing to heightened awareness among the people (Singh *et al.* 2021). After China, India is the highest onion producer with 22.81 million tonnes production from 1.22 million ha land (Singh *et al.* 2021). Exclusively, alliums including onion, garlic and shallots are contributing to more than 50% share among exported vegetable crops from India with earnings of $498 million during 2018–19 (http://agriexchange.apeda.gov.in/). Being second largest producer, Indian growers are getting considerably lower productivity compared to other countries like Korea, USA etc. The primary reasons of low productivity are cultivation of OPVs, locally grown seed with no proper quality control and lack of hybrids in the market (Khar and Singh 2020).

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Heterosis has been widely exploited in various crops that is manifested by the genetic expression of the developmental differences among hybrids and their respective parents. Onion hybrids exhibit higher heterosis over open pollinated varieties in terms of yield and agronomical characteristics (Singh and Bhonde 2011). Hybrids are quite popular among onion growers worldwide due to their high yielding ability with uniform bulb, morphological and maturity traits. This crop is highly cross-pollinated and is put in the category of crops showing steep inbreeding depression. Exploitation of heterosis breeding is the best alternate to enhance onion productivity in India. Worldwide, onion hybrids are released with the use of cytoplasmic male sterility caused by S-cytoplasm (CMS-S). The CMS-S was discovered in the pedigree Italian Red 13-53 (Jones and Emsweller 1936) and this is controlled by a single dominant nuclear *Ms* locus (Jones and Clarke 1943). Further, Berninger (1965) classified T-cytoplasm (CMS-T) in which fertility was controlled by two complementary and one independent gene (Schweisguth 1973). These research outcomes established the foundation of heterosis breeding and development of hybrids in US and other developed countries.

Availability of suitable inbreds is the primary and critical factor for heterosis breeding program and superior hybrid development in any crop. In case of onion, making inbred availability is not so easy due to its high inbreeding depression and biennial crop cycle which needs about 10-12 years. Till date, no commercial hybrid of onion is available at national level from public sector. However, IIHR Bengaluru released two hybrids (Arka Lalima and Arka Kirthiman) at state level that didn't gain much commercial popularity

among the farmers at national level. Thus, it is need of the hour to develop suitable inbred lines for heterosis breeding program in short time period. For development of inbred lines through accelerated approach, haploid induction is the best and efficient alternative. Hybrid onions are produced on a commercial basis in the USA, Netherlands, Korea, Japan etc. On the other hand, countries like India, are unable to explore the potential of heterosis and need to take it up proactively by providing more impetus and sufficient infrastructure towards hybrid development in public institutes. Keeping all these facts in view, this is the first review article, which is attempted to comprehensively review the hybrid breeding perspectives with respect to Indian scenario.

Production and productivity trend in India

For the last two and half decades, onion production has enhanced more than fivefold from 40.35 lakh tons (1994–95) to 228.19 lakh tons (2018–19) with the development of high yielding OP varieties by the public sector. After China, India ranks second in onion production having 18.7 t/ha productivity which is significantly lesser than other onion producing countries such as US, Netherlands, UK, Korea and China. In 2018–19, India exported about 21.83 lakh tons of onion and earned ₹ 3468.61 crores. States like Maharashtra, Karnataka, Gujarat, Bihar, Madhya Pradesh and Rajasthan are the major onion producing states of the country (http://nhrdf.org).

Foundation of onion research in India

Organized and systematized onion research in India was initiated during 1960 at Nashik and consecutively at IARI, New Delhi, IIHR, Bengaluru and National Horticultural Research and Development Foundation (NHRDF), Nashik in 1977 (Lawande *et al.* 2009). NHRDF played vital role by developing high yielding varieties and production technology. During early nineties, State Agricultural Universities (SAUs) and All-India Coordinated Research Project (AICRP) strengthened onion research in the country in the form of development of new varieties for various agro-climatic zones with refined agronomical practices. To boost productivity with specific focus on onion research, ICAR established National Research Center on Onion and Garlic (NRCOG) at Nashik in 1994 that was shifted to Rajgurunagar in 1998 and upgraded as Directorate of Onion and Garlic Research in 2009. ICAR institutes along with few SAUs such as MPKV Rahuri, PAU Ludhiana and CCSHAU Hisar are contributing towards onion research. In India, it was well emphasized to develop F_1 hybrids in short-day tropical onion during late nineties, but not much success has been achieved and is still an imperative and attention seeking aspect of onion research (Lawande *et al.* 2009). In 1948, first attempt to develop F_1 hybrids in onion was done by Sen and Srivastava (1957) using exotic male sterile line and indigenous genotypes as male parent but they did not get success due to stability issues of male sterile lines under Indian tropical conditions. To develop heterosis breeding program in Indian scenario, some of the critical breeding objectives are:

- Production of suitable and stable inbreds
- Identification of male sterile and their maintainers from local and commercial Indian population
- Exploitation of heterosis for enhancing productivity and wider adaptability
- Resistance/tolerance against various biotic and abiotic stresses
- Development of hybrids having desired quality and high storability with great potential to export

Male sterile cytoplasms in onion

N-cytoplasm: Normal (N) cytoplasm might have been developed from *Allium vavilovii* and is reported to be the progenitor of onion cytoplasm and this cytoplasm is predominant in occurrence in wild and domesticated *Allium* species of Central Asia (Havey 1997). In open pollinated onion populations, this type of cytoplasm is dominant with high frequency of dominant fertility restorer (*Ms*) alleles (Havey and Randle 1996, Gökçe and Havey 2006). The major reason is continuous selection of dominant against recessive alleles over several years and generations. Identification of maintainer lines having N*msms* genetic makeup from dominant (*Ms*) allelic population is complicated and time consuming, due to prevalence of dominant alleles in normal cytoplasmic populations (Havey 1993, Havey and Randle 1996) (Table 1).

S-cytoplasm: This type of cytoplasm was firstly acknowledged at University of California, USA by Prof. H A Jones in the onion population of Italian Red in 1925. The identified line named 13-53 acted as foundation of heterosis breeding in onion across the world. Molecular studies revealed that S-cytoplasm is of an alloplasmic origin (Holford *et al.* 1988). Further, molecular genetics revealed that the origin of S-cytoplasm is due to the increased genomic shift of *orf*725 gene with the decrease of normal *cox*I gene (Kim *et al.* 2009). Havey (1997) hypothesised that S-cytoplasm diversified by cultivation of male sterile population in Central Asia and Middle East. He hypothesised that S-cytoplasm might have been introduced into N-cytoplasmic population in antiquity. Due to its stability and simple inheritance of fertility restorer, this cytoplasm is the first and best choice for the breeders to develop onion hybrids globally (Havey 1993, Sato 1998).

T-cytoplasm: After the discovery of N and S cytoplasm, Berninger (1965) identified and isolated third type of cytoplasm in onion population from France, designated as CMS-T cytoplasm and characterized by Schweisguth (1973) for commercial utilization in hybrid seed production, particularly in European countries. The restriction enzyme polymorphism of T-cytoplasmic population did not differ with N-cytoplasm, so it was hypothesised to be developed from N-cytoplasm. This might be due to point mutation in mitochondrial genome and it is revealed to be of autoplasmic origin from N cytoplasm in *Allium cepa*.

Table 1 Important chronological events related to onion male sterility

Report/Event	Country	Reference
Identification of S cytoplasm in *Italian Red 13-53* and fertility controlled by single gene	US	Jones and Emsweller (1936), Jones and Clarke (1943)
CMS-T and controlled by 3 independent loci	France	Berninger (1965), Schweisguth (1973)
Galanthum cytoplasm	US	McCollum (1971)
PCR markers developed for distinguishing N, S and T cytotypes	South Korea	Kim *et al.* (2009)
Development of PCR based marker linked to *Ms* locus	South Korea	Bang *et al.* (2011)
Validation of SNPs for nuclear *Ms* locus	US	Havey (2013)
High-resolution linkage map of *Ms* locus	South Korea	Park *et al.* (2013)
Sequencing and annotation of onion chloroplast genome	US	Kohn *et al.* (2013)
Codominant marker in linkage disequilibrium with *Ms* locus	South Korea	Kim (2014)
A multiplex PCR marker, AcSKP1, for *Ms* locus detection	China	Huo *et al.* (2015)
FISH mapping of markers tightly linked to *Ms* locus	US	Khrustaleva *et al.* (2016)
PCR based markers validation in Indian short-day onion	India	Khar and Saini (2016)
Transcriptomic studies related to male sterility	China	Yuan *et al.* (2018)
'Y' cytoplasm	South Korea	Kim *et al.* (2019)

Table 2 Assessment of cytoplasm and *Ms* locus frequencies in short day Indian onion

Cytoplasm markers	Cytoplasm type		Nuclear markers	Ms locus		
	N	S		MsMs	Msms	msms
Sel. 121-1						
OSN	0.00	1.00	OPT	0.17	0.71	0.12
MKFR	0.00	1.00	jnurf13	0.00	0.33	0.67
accD	0.00	1.00	AcSKP1	0.00	0.54	0.46
			AcPMS1	0.00	0.33	0.67
Sel. 121-2						
OSN	0.12	0.88	OPT	0.21	0.46	0.33
MKFR	0.12	0.88	jnurf13	0.00	0.29	0.71
accD	0.12	0.88	AcSKP1	0.00	0.38	0.62
			AcPMS1	0.00	0.38	0.62
Pusa Red						
OSN	0.67	0.33	OPT	0.19	0.57	0.24
MKFR	0.67	0.33	jnurf13	0.05	0.09	0.86
accD	0.67	0.33	AcSKP1	0.05	0.18	0.77
Pusa Riddhi						
OSN	1.00	0.00	OPT	0.70	0.22	0.09
MKFR	1.00	0.00	jnurf13	0.00	0.00	1.00
accD	1.00	0.00	AcSKP1	0.00	0.00	1.00
Early Grano						
OSN	1.00	0.00	OPT	0.22	0.78	0.00
MKFR	1.00	0.00	jnurf13	0.00	0.00	1.00
accD	1.00	0.00	AcSKP1	0.00	0.09	0.91
Pusa Madhav						
OSN	0.83	0.17	OPT	0.22	0.64	0.14
MKFR	0.83	0.17	jnurf13	0.00	0.00	1.00
accD	0.83	0.17	AcSKP1	0.00	0.00	1.00

Source: Khar and Saini 2016

Further studies exhibited that S and T cytoplasms are of different origin (Holford *et al.* 1988, Singh *et al.* 2021). The molecular genetics revealed the CMS-T mitotype originated due to the genomic shift of *orf725* gene through increased copy number but without decrease of normal *coxI* gene (Kim *et al.* 2009). Organization of nucleotide sequences in the gene revealed that CMS-T cytotype was found to be recently originated from normal cytotype (Kim and Yoon 2010) (Table 2).

Onion heterosis breeding in India

Importance of heterosis or hybrid vigor has attracted plant breeders ever since the discovery of various mechanisms like male sterility, self-incompatibility by the scientists in crop plants. Globally, developed countries like US, UK, Netherlands, Japan, Korea exploited heterosis extensively to develop onion hybrids. Countries like India, being major onion producing country, still lag behind to exploit heterosis in onion crop. That is the primary reason for lesser productivity compared to other onion growing countries. Till date, no commercially popular hybrid from public sector has been released in India at national level. Focused research work on onion hybrid development has not gained pace in the past decade and needs to be prioritized. First attempt for hybrid onion development was recorded in 1948 by crossing exotic male-sterile lines with local landraces. The first hybrid namely VL 67 was released for Uttarakhand Hills in 1973 (Khar and Saini 2016). After the initial success, few attempts have been made by Indian scientists for identification and isolation of male sterile plants from locally grown cultivars. Male-sterile plants were identified from local cultivars like Niphad-2-4-1, Nasik White Globe, IIHR-20 and Pusa Red (Patil *et al.* 1973, Pathak *et al.* 1980, Pathak 1997).

Systematic work on hybrid onion was started after the establishment of IIHR, Bengaluru by Dr Pathak. His team took 33 onion varieties under study and found that only two varieties namely Arka Pragati (Indian variety) and Red Coral (exotic variety) possessed S cytoplasm (Pathak *et al.* 1980). This was the initial report on S-cytoplasm in Indian short-day onion having S cytoplasm. Correspondingly, Pathak and Gowda (1993) also tried to exploit exotic male sterile lines in their heterosis breeding program at IIHR, but they failed to get success owing to short photoperiodic conditions. Mani *et al.* (1999) exploited heterosis for various economic and agronomic traits, and released landmark OP variety VL Piaz-3 for hilly zones of Uttarakhand. Furthermore, Pathak (1997) obtained male sterile hybrids by attempting crosses between a male-sterile line and fertile lines. He observed that all the test crosses were sterile and opined that either all the fertile lines used in the crossing possess *ms* recessive loci or there is no control of nuclear genes on sterile cytoplasm. Based on his findings, he hypothesized that Indian cytoplasm was different from S and T cytoplasm and was indeed a new form of male sterile cytoplasm. Further, he observed that flowers having light green anthers were male sterile and dark green anthers were male fertile. These findings were in contrast to the results reported by Khar and Saini (2016). They recorded that male sterile flowers do possess light green, dark green or yellow anthers. Likewise, Saini *et al.* (2015) didn't observe any correlation among the male sterility and anther colour, but they observed irregular production of fertile pollen in male-sterile lines that might be because of higher temperature effects on pollen production. Our observations of male fertile and sterile flowers, anthers and pollen in the short-day Indian onion are displayed in the Fig 1.

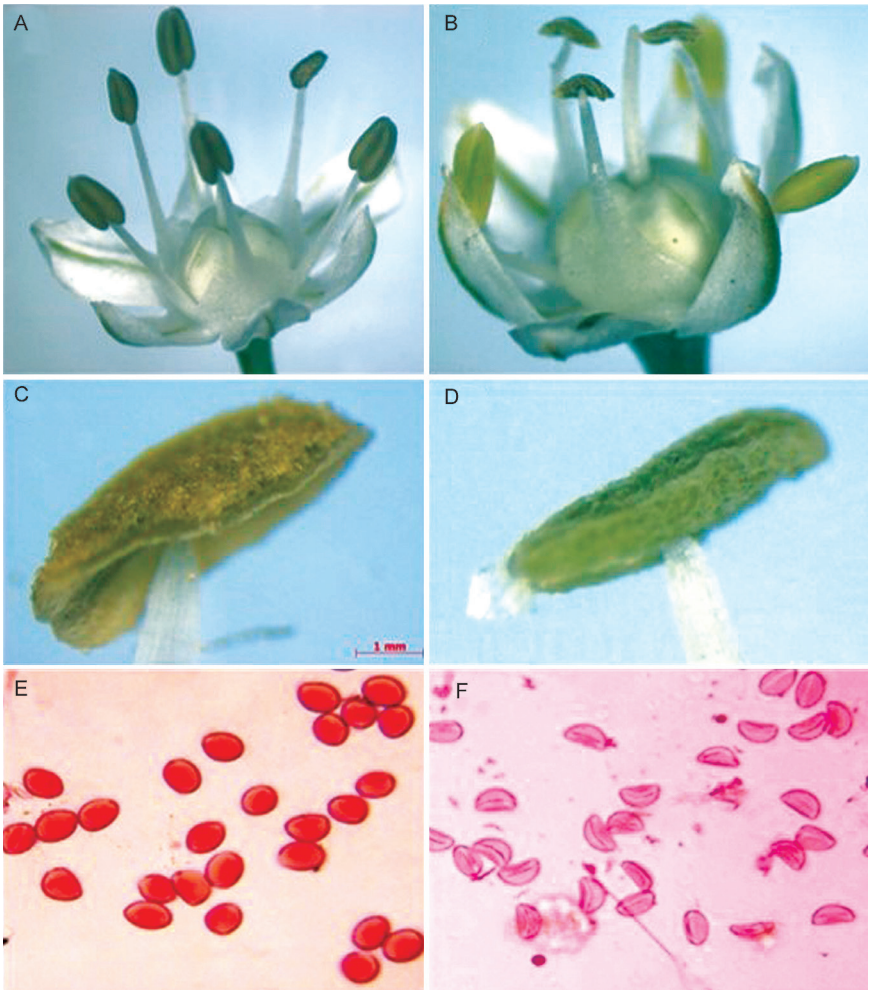

Fig 1 Morphology of onion male sterile and fertile reproductive organs. A) Fertile flowers, B) Sterile flowers, C) Fertile anthers, D) Sterile anthers, E) Fertile/viable pollen grains and F) Sterile/non-viable pollen grains.

IIHR Bengaluru released two F_1 onion hybrids namely Arka Kirthiman and Arka Lalima (Pathak 1997). These hybrids were unable to get commercial popularity and adoption among the onion growers at national level (Khar and Saini 2016). Manjunathagowda and Anjanappa (2020) identified male sterile lines from Indian onion population. They observed that male sterile flowers had longer style, narrow stigmatic knob, produced functionally non-viable pollens and perianths did not open fully. Light green to slightly yellowish translucent, shriveled, seldom empty anther without pollen grains with fused anther sacs were observed.

Molecular markers for identification of cytotype and fertility restoration

Globally, breeders prefer CMS-S cytoplasm for the production of onion F_1 hybrids since this cytoplasm is more stable with monogenic inheritance and less influenced by varied environmental conditions. Being biennial crop, developing hybrids is a cumbersome and tedious procedure because it demands 4 to 8 years to know cytoplasm type and *Ms* alleles through progeny testing (Havey 2000). Besides the above issue, manual emasculation is also problematic owing to very small and large number of flowers in the umbel. To overcome these issues, reliable and validated molecular markers are the ideal technique to reduce the lengthy and laborious conventional methods.

In India, preliminary report on use of molecular markers for identifying cytoplasm in local onion populations was reported during end of first decade of this century. Chaurasia *et al*. (2010) reported that Arka Kirthiman and Arka Pitambar cultivars had CMS-S cytoplasm. Havey (1993) endorsed that Indian onion accessions are N cytoplasmic except PI288272. Thereafter, Sheemar and Dhatt (2015) reported CMS-S cytotype in 3 Indian short-day populations, whereas other 3 populations possessed only N cytoplasm. A significant study was conducted by Khar and Saini (2016), to validate the already reported PCR based markers in order to determine the frequency of S or N cytotypes and *Ms* locus in Indian short day onion genotypes. They also correlated molecular observations with morphological traits linked with pollen fertility, viz. anther colour, pollen shape and pollen size visually and under microscope. They concluded that PCR markers used for detecting cytoplasm are robust and the marker *accD* was found to be better because of the ease of its use and amplification. On the other hand, no *Ms* locus linked marker showed complete linkage disequilibrium with *Ms* locus in Indian short day onion population (Table 2). It was elucidated that all the markers were equally good in determining the cytoplasm and all of them detected the same type of cytoplasm without any ambiguity. Use of PCR markers to carry out the genotyping of the *Ms* locus in open pollinated populations with certainty is of utmost importance towards speedy development of onion hybrids. Further, Khar and Saini (2016) pointed out the inconsistencies between molecular genotyping, field inspection and microscopic visualization and also revealed that *OPT1* marker is not in linkage disequilibrium with the *Ms* locus in short-day Indian onion population. Validated codominant markers, *jnurf13*, *AcSKP1* and *AcPMS1* also displayed discrepancies in linkage disequilibrium with the *Ms* locus.

Doubled haploid (DH) induction for inbred development

For heterosis breeding program of any crop, development of complete homozygous inbred lines plays a vital role. Several aspects like nature of crop, pollination behaviour, flower size and structure, breeding system, etc immensely influence development of homozygous lines. Comparatively, it is very easy to develop in self-pollinated crop as compared to cross-pollinated crops such as onion. This bulbous crop exhibits greater heterozygosity, outbreeding nature, biennial growth habit, protandry genetic mechanism and massive genome size (Havey 1993). High inbreeding depression in onion led to decline of yield due to reduction of bulb size after few generations of selfing (Villanueva-Mosqueda and Havey 2001). To overcome above-mentioned issues, DH development is an important biotechnological tool to speed up the inbred development. In onion, genotype, stage of ovule/flower development, pre-treatment, culture media and agronomical practices are profusely critical for achieving haploids. Irrespective of other crops, in onion, use of anthers has not been ideal, but gynogenesis has been preferred for haploid induction. Reports suggest that for haploid induction, genotype, day length, geographical origin, type and development stage of explant and cultural media and conditions (Alan *et al.* 2004, Jakše *et al.* 2010, Anandhan *et al.* 2014, Khar *et al.* 2018, Khar *et al.* 2019) are the fundamental and critical factors for the successful *in-vitro* gynogenesis.

For *in-vitro* culture of flower buds, the perfect stage is around 3-5 mm size that corresponds to 3 to 5 days earlier to anthesis (Alan *et al.* 2004). An efficient and simplified single step protocol consisting of entire flower bud culture on *in-vitro* induction medium until embryo stage developed is widely adopted by the onion breeders across the world. As per literature availability, major research work is carried out in long day compared to short day onion. In India, there are few research reports on haploid induction studies in short day onion (Anandhan *et al.* 2014, Mathapati *et al.* 2019). Khar *et al.* (2018) evaluated indigenous and exotic genotypes with different media combinations for haploid induction. They pointed out that indigenous OPVs and hybrids were more responsive compared to exotic genotypes and landraces. Gamborg's B5 medium fortified with 2 mg/l 2,4-D and 2 mg/l BA was found to be the best media for short day Indian onion for the haploid induction. Mathapati *et al.* (2019) elaborated that there was no effect of hormones like Kinetin, meta-topolin and thidiazuron on embryo induction. These reports are the preliminary studies that have opened avenues to speed up inbred development that is a foundation for heterosis breeding in Indian onion. In this aspect, more research attention and focus is required to standardize reproducible and efficient protocol for rapid haploid induction by studying and identify the optimum

medium and genotypic specific response.

Hybrid seed production

Hybrid seed production of onion involves male-sterile line as a female parent with a genetic constitution of sterile (S) cytoplasm and homozygous recessive *Ms* locus (*msms*) and a male fertile line known as maintainer line or B lines having normal (N) cytoplasm and a homozygous recessive *Ms* locus (*msms*). Hence, the sterile parent (A line) will have a genetic constitution of *Smsms* and a maintainer line (B line) will have a genetic constitution of N*msms*. The B line is used to maintain the sterile line. For hybrid seed production, 'C' line, a male fertile line, with normal (N) cytoplasm and any of the genetic constitution at *Ms* locus (*MsMs*/*Msms*/*msms*) can be used. Both A lines and B lines are isogenic and are developed by backcrossing for 4-5 generations and C lines are developed by inbreeding of superior male sterile lines for at least two generations and then sibmating for two generations to achieve uniformity. In onion, the main part to be used as vegetable is bulb, it is immaterial whether the C line is maintainer or restorer line. In case of crops, where seed is the economical part, a restorer line is needed. Here, we are interested in C line which is diverse from the A line so that heterosis in terms of yield and productivity is achieved.

Conclusion

Development of onion hybrids is essential for the Indian farmers to boost country's agricultural economy and doubling farmers' income since this crop has great potential for the export. Schematic and organized work on the inbred development through haploid induction in short day onion cultivars is very important to augment the heterosis-breeding program. Induction of haploid depends on various factors and genetic architecture plays a vigorous role. Some research groups are trying their hard to achieve it. The next most critical point is identification of *Ms* locus and different types of cytoplasms in short-day onion population at molecular level. Development and validation of molecular markers in linkage disequilibrium with *Ms* locus is required to supplement and speed up onion breeding program in the country. Marker assisted selection (MAS) has potential advantage over traditional breeding methods for accelerated breeding of hybrid onions. In future, onion breeders need to be focus much on the identification of potential and polymorphic codominant molecular markers (SSR, SNPs) for genetic variation and identification of novel genotypes for further breeding program. Comparing with other crops such as cereal, tomato and pulses, molecular studies and genomics-assisted breeding in onion is far behind. Keeping in view, the demand and socio-economic importance of this bulb crop, we should instantly need to opt for various contemporary and up-to-date *omic* tools comprising of genomics, transcriptomics, proteomics and metabolomics for the study of complex traits at molecular level. Development of genomic resources is imperative to speed up the breeding and genetic improvement work for sustainable production to meet out the soaring demand of onion at national and international level.

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