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Short Communication

Variation in gynogenic potential for haploid induction in Indian short-day onions

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Abstract

Gynogenic haploid induction was tried in 14 Indian short-day onion varieties. Variation in gynogenic potential was observed (0.9 to 4.5 per cent) between genotypes. The highest frequency of gynogenic plants was in Bhima Shubhra (4.5 %) and the lowest in Bhima Shweta (0.9 %). Accumulation of mutant alleles likely contributed to the low gynogenic frequency. Time to embryo sprouting varied between 40 to 98 days after inoculation. Highest range in time of sprouting was observed in Bhima Shubhra, from 51 to 98 days. Non-haploid regenerants were also observed among gynogenic plants and their gynogenic origin was confirmed by SSR markers. Parents with good gynogenic potential can be utilized for introgression breeding in onion where a reduction in the breeding cycle is beneficial.

Key words: *Allium cepa*, inbred, ploidy, homozygous, gynogenesis

In onion, gynogenic induction is a method of choice for haploid regeneration through culturing isolated flower buds [1-3]. Gynogenic response varies among genotypes of different geographical origin, indicating the influence of genetic factors [4-7]. For instance, many American long-day genotypes have high gynogenic potential (22.6% in Rocket F_1) and European and Japanese accessions were less responsive to induction [3]. Gynogenic regeneration among different *Allium* spp. is also variable, with shallot-type onions (*Allium cepa* var *aggregatum*) having the highest gynogenic potential of 55.2% [8]. In other species such as leek (*Allium porrum*), chive (*A. schoenoprasum*), *A. altaicum* and *A. fistulosum*, though embryo formation occurs, but there is no regeneration [9]. In an inter-cross population of *A. cepa* x *A. royalei*, there was little gynogenic regeneration in early generations when compared to *A. cepa* and it improved in later backcross populations indicating, again, the influence of genetic factors [10].

Though there were many reports on gynogenesis in onion from genotypes of different geographical origin and photoperiodic behavior, but report on Indian group is limited. Therefore characterizing Indian genotypes for gynogenic potential becomes imperative, so that the genotypes may be useful for developing mapping population, breeding programmmes and also useful for genetic studies on gynogenesis. In the present study, variation in gynogenic potential and gynogenic induction of haploids in Indian short day onions were investigated.

Fourteen commonly cultivated varieties were selected for the study (Table 1). Flowers buds, three days before opening, were collected, surface washed with Tween 20 at 0.1% and surface sterilized with 70% ethanol for three minutes and 2% sodium hypochlorite for 15 minutes. Then the buds were washed with sterile distilled water, air dried and cultured on induction media (B5+ BAP (2mg/l) + 2-4-D (2mg/l) + 7.5 % sucrose). The number of gynogenic plants emerged was recorded and presented in terms of percent emergence and time of emergence. The ploidy of gynogenic plants was evaluated by cytology and flow cytometry (GeneOmbio, Pune, India). The gynogenic diploid were

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Table 1. Gynogenic potential among Indian short day onion varieties

Genotypes	No. of explants	No. of regene-rants	Gynogenesis (%)
Agri Found Rose	891	17	1.9
AgriFound Dark Red	200	4	2.0
Arka Kalyan	189	6	3.2
B780	295	10	3.3
Bhima Kiran	2300	65	2.8
Bhima Red	2000	38	1.9
Bhima Shakti	1250	26	2.1
Bhima Shubhra	441	20	4.5
Bhima Shweta	3800	34	0.9
Bhima Super	1000	35	3.5
N-2-4-1	500	15	3.0
Pusa White Round	286	4	1.3
RLKM-1	165	5	3.1
W514	165	3	1.8

evaluated with SSR markers. Amplification was carried out with 25µl reaction containing DNA 50 ng, $MgCl_2$ 1.5mM, dNTPs-0.2mM, primers 0.02mM each, and Taq DNA polymerase 1U (Merck). PCR conditions of 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 52-59 °C for 30s, and 72°C for 30s. The amplicons were resolved on 6% PAGE and imaged for scoring. Data were analyzed using the statistical package SAS 9.3 for the experiment laid in a completely randomized design. The data on genotypic potential for gynogenesis were analysed by χ^2 test.

Gynogenic induction was observed in all the accessions evaluated. The gynogenic plants emerging from matured ovaries (Fig. 1) were characterized by the presence of roots and shoots. Gynogenic frequency, evaluated on similar media, varied from 0.9 to 4.5 percent revealing a significant difference in gynogenic potential between the varieties evaluated (Table 1). The variety Bhima Shubhra recorded the highest frequency (4.5 %) while Bhima Shweta recorded the lowest (0.9 %). The gynogenic potential of the short days accessions was lower than that of reported for long day types such as the American long day genotypes which are reported to have a high gynogenic potential [3]. Low regeneration frequency can be attributed to poor gynogenic efficiency and population structure [7] where defective mutant alleles might have been the active contributors. In the present study, albinos (Fig. 2), rootless and leafless plantlets

Fig. 1. Gynogenic regenerant emerging from ovary cultures

were observed during regeneration and these defective plantlets were lost during culturing. Percentage of defective plantlets also varied among the genotypes, there were upto 60% in Bhima Shubhra and Bhima Super. Possibly the defective alleles could have contributed to reduction in the gynogenic potential. A second cycle of regeneration may improve the efficiency for better gynogenic potential.

Time to embryo sprouting varied from 40 to 98 days after inoculation. Early emergence was observed

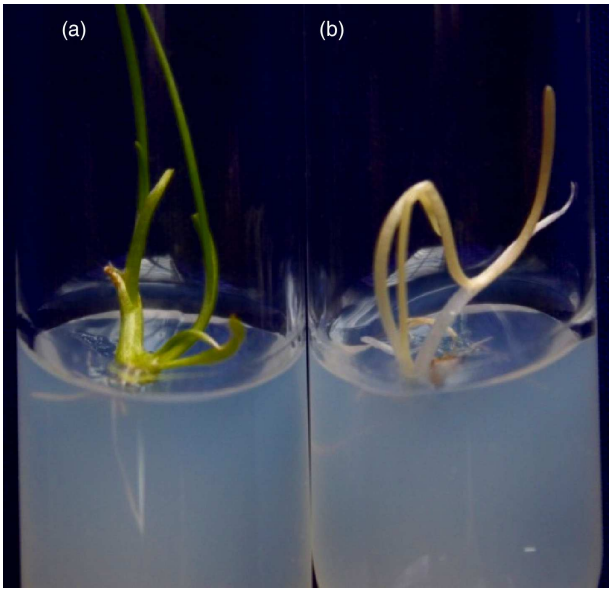

Fig. 2. An albino plantlet regenerated through gynogenesis. a: Normal, b: Albino

in variety AgriFound Rose and average time to sprouting was around 63.8 days. Maximum variation in time of emergence was in Bhima Shubhra, ranging from 51 days to 98 days. Average effect over all varieties time to sprouting was 65 days. Jakse *et al*., [6] reported that emergence of gynogenic plants could be between 46 to 152 days. Addition of spermidine (0.1mM) to the media hastened the time to emergence in the genotype Sefid-e-Kurdistan from 124 days to 62 days, whereas Sefid-e-Neishabou didi not respond to the addition of spermidine [11], indicating the possible influence of genotype on time to emergence. Yearly variation, too, play a role in the emergence of embryo [7].

Ploidy analysis was carried out through cytology and flow cytometry. Among the 59 plants analysed by cytology, 40 percent of gynogenic plants were found to be haploid, 52 percent were mixoploid and the rest were diploid. Among the 21 plants analysed through flow cytometry, 15 were haploid while the rest were diploid. Non-haploid gynogenic plants like diploids, triploids and teteraploids were observed in other studies too [7, 12] but at very low frequency up to 20 percent. Five SSR primers showing polymorphism in the parental population were used to test for the presence of a single allele. Among the gynogenic haploids only single allelic forms were observed as expected. The gynogenic origin of diploids was confirmed by presence single allelic form. Among the 7 plants evaluated, 5 were homozygous for the loci thus confirming the gynogenic origin (Fig. 3).

In the present study, we have evaluated the gynogenic potential of Indian short day onion varieties. Though the gynogenic potential was low, compared to long day varieties, there was considerable variation in gynogenic potential among the genotypes evaluated. The study provides a method for the gynogenic induction of haploids in Indian short day onion group and thus could be of use for developing doubled haploids for hybrid development programmes.

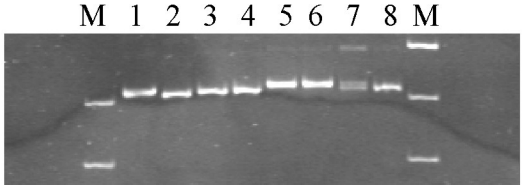

Fig. 3. SSR analysis for homozygosity in gynogenic diploids . Lane M: 100 bp marker 1- 7 Gynogenic Diploids (multiple bands in lane 7) 8- gynogenic haploid

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