Breeding Mediated Improvement of Mungbean [*Vigna radiata* (L) Wilczek] for Salt Tolerance

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Abstract

Mungbean is an important economically friendly grain legume. In the present study, previously selected salt resistant genotypes (EC 528960 (wild) and JP31300 (cultivated) of mungbean were tried to make cross with highly salt sensitive cultivar i.e. IC10492 of mungbean. The obtained inter and intra-specific hybrids of two different types of crosses i.e. IC10492(♀) x JP31300(♂) and IC10492(♀) x EC528960(♂) were assessed for morphological characterization and hybrid purity using azukibean specific SSR markers. The F_1 hybrids inherit the banding pattern similar to the male parent ascertaining the purity of the hybrids. The morphological characterization showed that germination potential (%) was observed ≥90% on third day, flowering was synchronous and days to flower were 30.5 days in all the F_1 hybrids. The F_2 population can be used as mapping population or for development of recombinant inbreeds lines (RILs). Present investigation suggests that breeding mediated introduction of salt resistance in mungbean is an efficient method. This method may help in development of improved variety of mungbean for saline soil.

Keywords: mungbean, breeding, salt tolerance, microsatellite markers (SSRs), hybrid purity

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek), is an economically important, valuable and highly nutritious food crop. It is rich source of protein, vitamins and minerals for the vegetarian diet of Asians where nearly 90% of the world's mungbean is produced annually (Tomooka et al. 2002). This crop is self-pollinated diploid crop with 2n = 2x = 22 chromosomes and a genome size of 579 Mb. Nitrogen fixing ability and short duration (55-90 days) makes it important in various cropping systems (Somta and Srinives, 2007). It is consumed as "dhal", which is soup porridge and combination of mungbean and cereal in diet which provides a more balanced amino acid profile. It is used for bean sprouts, starch noodles, green pods as peas in cooking, mungbean soup and deep fried patties of different kinds throughout the world. Mungbean seeds and soup are also a rich source of alkaloids, coumarin and phytosterin that play an important role in promoting the physiological metabolism of human beings and animals. India is the largest producer and consumer of mungbean and accounts for about 65% of the world acreage and 54% of the world production of this crop. It is the third most important pulse crop in India, occupying nearly 3.72 million ha area with 1.56 million tons production (Ali and Gupta, 2012).

Among the various abiotic stress factors, salinity stress is considered as major restraint in its production where more than 70% yield loss may occur even under mild stressed conditions (Saha et al., 2010). High accumulation of Na^+ ion causes

adverse effects on activities of cytosolic enzyme, photosynthesis, metabolism, and potassium ion nutrition results in uncoupling of major physiological and biochemical processes (Jacoby et al., 1999; Arora et al., 2002; Srivalli et al., 2003). A huge proportion of agricultural land in the world is affected by salinity which is increasing continuously day by day (nearly 1% per year). The increased salinity of arable land is expected to have devastating global effects, resulting in up to 50% land loss by the middle of the twenty-first century (Mahajan and Tuteja, 2005). More than 45 million hectares (M ha) of irrigated land which accounts to 20% of the total land has been damaged by salt worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in the soil (Munns and Tester, 2008). Despite the enormous significance of mungbean, very little work has been reported for its genetic improvement by identifying and breeding cultivars for saline soil (Singh et al., 2011). The low productivity of mungbean highlights the need to boost up the productivity of this agronomically valuable food grain legume to meet the nutritious food demands of the geometrically increasing population by exploiting scarce natural resources more efficiently. The development of salt tolerant cultivars is the most promising approach to reduce the lethal effects of soil salinity on crop production (Epstein et al., 1980). Genetically diverse germplasm resistant to salinity stress within *Vigna* genotypes could be more convenient to study the mechanism governing salt tolerance and for the delivery of genetic resources for salinity in breeding program (Win et al., 2011).

Keeping the importance of all these facts in mind, the present study was undertaken to genetically improve the mungbean for saline areas with the following objectives: To make crosses between highly salt resistant and salt susceptible wild relatives and cultivated mungbean genotypes and to confirm the purity of the F1 hybrids developed for salt tolerance.

Materials and Methods

Plant Material and SSRs Markers

The seeds of IC10492 & EC528960 were procured from core collection at National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India and the seeds of JP31300 were procured from GeneBank, National Institute of Agrobiological Sciences (NIAS), Japan (Table 1). The azukibean specific SSRs (CEDG149 & CEDG051) were used for the assessment of F_1 hybrids (Table 2).

Hybridization of Salt Susceptible and Resistant Genotypes

Salt resistant [JP31300 (♂) & EC528960 (♂)] and salt susceptible [IC10492 (♀)] parental genotypes of mungbean were grown in separate rows with proper spacing. Hybridization was performed using hand emasculation and pollination method **(**Boling et al. 1961**).** The experiment was carried out in the field plot at herbal garden. The flowers of the female parent were emasculated without any damage to the receptive stigma. Mature anthers were collected from the male parent and dehisced on the stigma. Thereafter, the flower bud was covered with cotton and tagged properly for identification. The neighbouring non-crossed flowers were removed. The crossed pods were harvested after maturation. The F_1 hybrids [IC10492 (♀) x JP31300 (♂)] and [IC10492 (♀) x EC528960 (♂)] and their respective parents were raised in Green House at M.D.U., Rohtak.

Table 1 Details of the parental lines used in this study.

Sr. No.	Name of the genotype[1/]	Response for salt stress	Plant source	Parent type	Genetic resource
P_1	IC10492 (C)	Highly salt susceptible	*Vigna radiata*	Female	NBPGR, New Delhi, India
P_2	JP31300 (C)	Highly salt resistant	*Vigna radiata*	Male	GeneBank, NIAS, Japan
P_3	EC528960 (W)	Highly salt resistant	*Vigna luteola*	Male	NBPGR, New Delhi, India

[1/] C = cultivated genotype & W = wild genotype

Table 2 Details of the azukibean specific SSRs used for the assessment of hybrid purity.

Primer	Forward sequence	Reverse sequence	Motifs	Tm	Linkage group
CEDG051	AAACATACCCCTGGCAGTTCC	TTCTGACCTAAGAAAGAGCCTGG	(AG)12	60	1
CEDG149	GGCTGAAGGTGATGACAGAAG	GGCACTGGTTTTCTAAGGTTGTTG	(AT)12(AG)16	60	1

Assessment of Hybrids Purity Using SSR Markers

The genomic DNA was extracted from young leaves of 25 days old F_1 hybrid plants and their respective progenitors [IC10492 (P_1), JP31300 (P_2) & EC528960 (P_3)] using Gene Elute Plant genomic DNA Extraction Kit (Sigma, USA) according to the instructions of the manufacturer. The purified DNA was diluted appropriately (30 ng μL^{-1}). The PCR reaction mixture was prepared in 10 µl volume containing template DNA (30 ng), 10X *Taq* Buffer B (Banglore Genei, India) in final concentration of 1X, forward and reverse primer (5 µM; Sigma, USA), dNTPs (2.5 mM; Fermentas, USA) and 0.025 units of Taq DNA polymerase (5U μL^{-1}, Bangalore Genei, India). The PCR was run on thermo cycler (Applied Biosystem Gene AMP PCR System 9700). The PCR program included initial denaturation at 94°C for 30 sec followed by 40 cycles each consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 1 min, elongation at 72°C for 30 sec. Final extension was carried out at 72°C for 10 min. The PCR products were visualized by agarose gel electrophoresis. The 100 bp DNA ladder (50 ng μL^{-1}; Fermentas, USA) was used as molecular weight marker. DNA banding patterns of hybrids were analyzed and assessed for the purity.

Other Characteristics Observed for the F_1 Hybrids

The seeds viability in terms of seed germination percentage, seed size appearance and days to flowering were recorded in the hybrid plants. At maturity, the pods containing F_2 seeds were harvested and stored separately.

Results and Discussion

Hybridization of Salt Susceptible and Resistant Genotypes

The result showed that hand emasculation and pollination based crossing produced successful pod set. All selected male and female parents were compatible with each other. The salt tolerant cultivar JP31300 was highly compatible with the salt susceptible cultivar IC10492 as compared to salt tolerant wild EC528960 genotype. The cross [IC10492 (♀) x JP31300 (♂)] produced more number of pods with good seed filling. This showed that the pollen-grains of JP31300 cultivar were more fertile and viable than wild genotype EC528960. This may be due to species variations or difference in the flowering period of wild and cultivated mungbean. Shrivelled or immature seeds were also obtained in few crosses of IC10492 (♀) x EC528960 (♂) which showed non-viable or sterile hybrids. The number of F_1 seeds in the hybrid pods varied from 1 to 5. The cross over frequency has showed variations for each cross which ranged from 2.97 to 3.48% with an average of 3.23% (Table 3).

Assessment of Hybrids Purity Using SSR Markers

The azukibean specific SSR primers successfully amplified the DNA of hybrid plants. The DNA band pattern (Figure 1 and Figure 2) showed that the F_1 hybrids inherited the DNA banding pattern similar to the male parent which confirmed their true hybrid status.

The SSR (CEDG149) produced reproducible band of 188 bp in male parent JP31300 (salt tolerant) however it was absent in female parent IC10492 (salt susceptible). All F_1 hybrids (except 3M) of cross between IC10492 (♀) and JP31300 (♂) showed PCR product similar to the male parent which verified the F_1 plants as true hybrids (Figure 1).

The SSR (CEDG051) produced reproducible band of 238 bp in male parent EC528960 (salt tolerant wild) however it was absent in female parental cultivar IC10492 (salt susceptible). All F1 hybrids (except plant No. 19M and 12M) of cross between IC10492 (♀) and EC528960 (♂) showed

Table 3 Other characteristics of F_1 hybrids measured.

Sr. No.	F_1 hybrids and cross type	Number of F_1 seeds taken	Number of F_1 seeds germinated	Germination (%)	Average days to flowering (DAS)	Seed size	Cross-over frequency obtained
1	IC10492♀X JP31300♂ (Intra-specific)	10	9	90%	32	Bold	3.48
2	IC10492♀X EC528960♂ (Inter-specific)	6	6	100%	29	Bold	2.97
			Average	95%	30.5		3.23

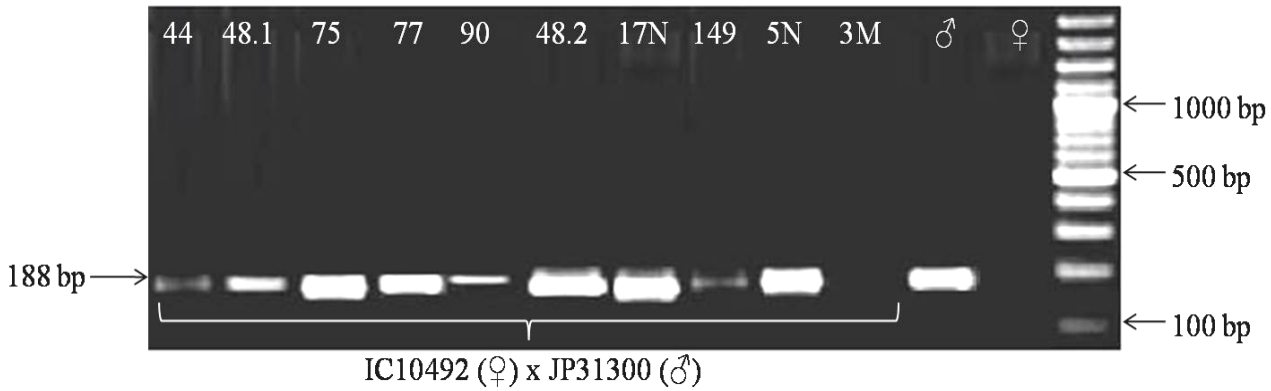

Figure 1 Banding pattern obtained in F1 hybrids and their respective parents on agarose gel electrophoresis using azukibean specific SSR marker (CEDG149): Lane 1= Marker (100bp), Lane 2= IC10492 (female parent), Lane 3= JP31300 (male parent), Lane 4 to 13= F1 hybrid plants IC10492 (♀) x JP31300 (♂)].

the presence of PCR product similar to the male parent which verified the F_1 plants as true hybrids (Figure 2).

Other Characteristics Observed for the F_1 Hybrids

Viability of the F_1 hybrid seeds [IC10492 (♀) x JP31300 (♂)] & [IC10492 (♀) x EC528960 (♂)] was measured in form of seed germination percentage. Out of the 16 F_1 seeds, 15 seeds were germinated on third day. The seeds germination ranged from 90% to 100% with an average value of 95%. The F_1 seeds were bold in appearance. The hybrid plants took 29 to 32 days for flowering with an average of 30.5 days after sowing (DAS) (Table 3). The F_2 seeds produced from each F_1 hybrid were collected and stored properly at 4°C for further study in future. The present results are in accordance to earlier findings on F_1 seed germination and flowering (Sehrawat et al., 2013).

Discussion

In present investigation, the hand emasculation and pollination based crossing (Boling et al., 1961) has produced successful crossed pods with good seed set in both intra and inter-specific crosses. The results are in accordance with the earlier findings (Singh and Malhotra, 1975; Ahuja and Singh, 1977; Park and Yang, 1978; Parida and Singh, 1985; Egawa et al., 1990; James et al., 1999), who reported 20 to 60% pod set with the same procedure. The cross over frequency was significant and similar to earlier findings (Van Rheenen, 1964; Bhadra and Shill, 1986). Shrivelled or immature seeds in some crosses [IC10492 (♀) x EC528960 (♂)] were found non-viable or sterile. The results are similar to findings of some earlier reports (AL-Yasiri and Coryne, 1966; Renganayaki, 1985; Chen et al., 1989; Ganeshram, 1993; Adinarayanamurthy et al., 1993; Uma Maheshwari,

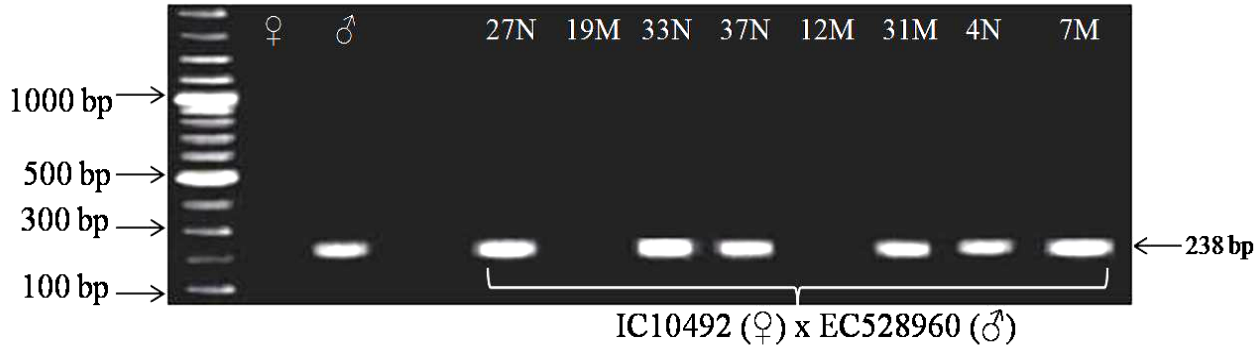

Figure 2 Banding pattern obtained in F1 hybrids and their respective parents on agarose gel electrophoresis using azukibean specific SSR marker (CEDG051): Lane 1= Marker (100bp), Lane 2= IC10492 (female parent), Lane 3= JP31300 (male parent), Lane 5 to 12= F1 hybrid plants IC10492 (♀) x EC528960 (♂)].

2002) which reported hybrid weakness, inviability, lethality and sterility as natural mechanism for maintaining the integrity of related species. The azukibean specific SSRs were found efficient in identification of true hybrids developed for salt tolerance. The F_1 hybrids obtained in this study inherited the DNA banding pattern similar to their male parents which confirmed them as true hybrids. Bianco et al. (2010) have reported use of ISSR markers for the confirmation of true F_1 hybrids of artichoke. Amplification of specific locus from genome of one species with help of primers designed from another species depends on the evolutionary distance between the two species and also on the rate of evolution of genome region in which the primer sequences are located (Souframanien and Gopalkrishna, 2009). The sequence conservation during evolution is the basis of cross-species utilization of SSR primers in genotyping across species and genera (Decroocq et al., 2003). The SSR markers are important tool for germplasm analysis, accessing genetic diversity, purity test of hybrids and linkage mapping of mungbean (Tangphatsornruang et al., 2009). Microsatellite markers can be used for genomic studies, marker assisted breeding in mungbean.

Conclusions

In present studies, the selected mungbean genotypes showed significant compatibility for crossing. The F_1 hybrids of mungbean were developed successfully for salt tolerance. Almost all hybrid seeds showed good germination and normal growth. The azukibean specific SSR markers were found efficient to assess the genetic purity of F_1 hybrids. The F_2 seeds can be used as mapping population for the construction of linkage map. Recombinant inbred lines can be produced from the F_2 seeds. Further generations (F3, F4 and so on) can be screen for salt tolerance. This study suggested that resistance for polygenic trait can be introduced into mungbean or other sensitive crops via breeding and the markers may facilitate this approach as marker assisted breeding.

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