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Identification of salt resistant wild relatives of mungbean (*Vigna radiata* (L.) Wilczek)

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ABSTRACT

Twenty two wild relatives of mungbean belong to seven Vigna species were screened for salt tolerance under two salinity levels (control and 250mM NaCl) with five replications per treatment. The experiment was carried out in earthen pots containing soil, sand and farmyard manure (1:2:1 ratio) and lined with polythene bags in complete randomized block design (RBD). The investigated genotypes exhibited significantly variable response towards salt stress. The adverse effects of salinity on plant growth, branching, leaf size and color, necrosis and chlorosis symptoms were observed visually by taking photographs at regular intervals during the crop season. The symptoms of major biotic stress encountering with salinity stress i.e. yellow mosaic virus were also observed. Less reduction in the observed traits in the genotypes EC528960 and TCR86 indicated their efficient adaptability under saline environment and can be considered as salt tolerant as compared to the genotypes which exhibited reverse response. Salinity caused ≥80% up to 100% loss of yield in most of the genotypes that indicated their high susceptibility for salt stress. The identified salt resistant genotypes EC528960 and TCR86 can be effectively used as a source of major genes or traits that can be introgressed into the susceptible genotypes as mungbean or other related week crop by breeding for their genetic enhancement for saline regions.

Keywords: Mungbean, salinity stress, salt tolerance, screening

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INTRODUCTION

Mungbean belongs to the genus *Vigna* subgenus *Ceratotropis* and is native to Asian tropical regions with greatest magnitude of genetic diversity [1, 2]. The genus *Vigna* is composed of more than 150 species originating mainly from Africa and Asia [3] which include a total of seven cultivated species of as cowpea (*Vigna unguiculata* (L.) Walp.), bambara groundnuts (*Vigna subterranea* (L.) Verdc.), mungbean (*Vigna radiata* (L.) Wilczek), urdbean (*Vigna mungo* (L.) Hepper), azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi), moth bean (*Vigna aconitifolia* (Jacq.) Marechal), and rice bean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi). The progenitor of mungbean, *V. radiata* var. *sublobata* is widely distributed from West Africa to Northern Australia and Papua New Guinea [4].

Mungbean is an important eco-friendly food grain leguminous crop of dryland agriculture with rich source of proteins, vitamins, and minerals [5]. It is a self-pollinated diploid crop with 2n = 2x = 22 chromosomes and a genome size of 579 Mb. The seeds of mungbean contain an average of 26% protein, 62.5% carbohydrates, 1.4% fat, 4.2% fibers, vitamins and minerals. It is consumed as "dhal", which is soup porridge combined with cereal or other traditional cuisines. Worldwide, mungbean is used for bean sprouts, starch noodles, green pods as peas in cooking, mungbean soup and deep fried patties of different kinds. This crop can be used for both seeds and forage because it

can produce a large amount of biomass and then recover after grazing to yield abundant seeds. Mungbean play an impotant role in sustainable agriculture production due to symbiotic association of roots and Rhizobia which reduce the cost for nitrogen fertilizers [6]. Short life span (65-90 days) and ability to restore the soil fertility makes it valuable in various cropping systems generally after rice crop. 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 however the productivity is still low 400 kg/ha [7, 8].

The agricultural productivity of this crop is drastically limited because of adverse effects of various abiotic and biotic stress causing factors. The agricultural productivity of this crop is drastically limited due to salinity stress: one of the most appalling environmental factors for the most salt-sensitive legume crops resulted >70% yield loss even under mild stress conditions in arid and semiarid regions [9]. The arable land is continuously transforming into saline (1-3% per year) either due to natural salinity or induced by human and the increased salinity is expected to have devastating global effects, resulting in up to 50% land loss by 2050 [10, 11]. Because of continuous use of traditional methods of irrigation (rain water, tube wells, and canals), the harmful ground water rises up and damaging the upper soil level utilized for agriculture. Similar performances would entirely jeopardize the agricultural capacity of fertile soil in salinity prone areas which may result in rigorous effects. Salt stress inflicts considerable adverse effects on physiology and performance of the crop plants which ultimately lead to plant death as a consequence of growth arrest and metabolic damage [12]. However; the intensity of adverse and injurious effects of salinity stress depends upon the plant species, nature, concentration, duration, stage, and mode of salt application to the crop. Evaluation of the germplasm in saline environment will certainly provide suitable material as a resource of agronomic traits or genes that can be introduced in the salt sensitive legume crops as mungbean by breeding [13].

Salt tolerance is complex genetically and physiologically and is also influenced by many plant, soil, and environmental factors and their interrelationships. It is a developmentally regulated, stage-specific phenomenon, so that tolerance at one stage of development may not be correlated with tolerance at other developmental stages [14]. The wild relatives of crop species possess greater genetic diversity than their related cultigens and are considered as source of important genes for improvement of agricultural productivity of mungbean [15, 16]. By considering the importance of all these aspects, the present study aimed to identify the most salt tolerant wild relative of mungbean that can be used as parent to introduce genes or valuable traits providing salt tolerance in mungbean via breeding.

MATERIALS AND METHODS

Plant material

Seeds of twenty two genotypes constituting wild relatives of mungbean available at core collection, National Bureau of plant Genetic Resources, Pusa Campus, New Delhi-110012 were used for the study (Table 1).

Salt solutions

Two salinity levels of 0 mM NaCl (Control) and 250mM NaCl were prepared by dissolving sodium chloride in the water used for irrigation for imposing stress in wild relatives of mungbean. The control treatment was without sodium chloride.

Screening for salt tolerance

Seeds of all genotypes were sown in 30 cm earthen pots (30 x 30 cm) containing 10 kg of soil, sand, and farmyard manure in 1:2:1 ratio, respectively. The experiment was carried out under an artificial rain shelter or hut made up of bamboos and polythene (PVC) with approximate 99% transparency or visibility so that the plants could absorb the sufficient light for photosynthesis and growth and the other contaminating or stress causing factors like natural rain, strong wind etc. interfering with the salinity treatment could be avoidedin the glass house. The removal of the weeds was done by hand regularly. The plants were thinned to 5 plants per pot after one week of seed germination. The salt solution of 250mM NaCl solutions of was applied to the plants i.e. 2.5 litre/kg of soil, after the emergence of fully expanded primary leaves in all the genotypes for imposing salinity stress. The plants applied with equal volume of water (without salt) were used as control (C). Scheduled routine of irrigation was practiced for both the control and the salt treated pots throughout the crop growth period. The effect of salt stress on plant growth, leaf size and color, necrosis and chlorosis symptoms was observed visually and the photographs were taken at regular intervals of time (15, 30, and 45 DAT) for the comparision of control and salt treated plants.

Fig. 1 Effect of salinity stress on plant growth, number of trifoliates, number of branches, leaf size and color (A: control plants and B: salt treated plants)

RESULTS AND DISCUSSION

Effect of salinity on plant growth

Salinity caused significant reduction in plant growth as length, number of trifoliates, secondary branches, leaf expansion area or size, and variation in leaf color (pale green) in all the wild relatives compared with their respective control plants during all growth stages (vegetative, flowering, and pod-filling) (Fig. 1). However; the genotypes EC528960 and TCR86 showed less reduction in these traits that indicates their considerable adaptability in stressed conditions up to the harvest of the crop (Figs 2 and 3). Salt stress caused low intra-cellular water potential and water scarcity around the root zone due to which roots failed to absorb sufficient water and nutrients for adequate plant growth [17]. Salinity affects the plant growth by inducing osmotic stress and ion toxicity which further interfere with mineral nutrients and caused alteration in various signaling processes (physiological, biochemical and molecular) and related metabolism pathways [18, 19]. Growth inhibition by salt stress may be due to the diversion of energy from growth to maintenance [20, 21].

Salinity induced other adverse changes

The salt stressed plants were observed with highly pronounced chlorosis and necrosis symptoms due to loss of chlorophyll contents that further affect the photosynthetic efficiency of the plants (Figs. 4 and 5). Symptoms of yellow mosaic virus were also observed hugely in salt stressed plants but variations were detected in different wild relatives. The plants of some genotypes were severely affected due to YMV but some showed less infection (Fig. 6). Salinity stress caused swelling of membranes in chloroplasts of sensitive plants which affects their chlorophyll content, or due to excess ion (Na^+ and Cl^-) in leaves which induced loss of chlorophylls [22, 23, 24].

Fig. 2 Salt resistant wild genotype EC528960 *V. luteola* at early (A& B) and late vegetative stage (C & D) where A & C: control plants; B & D: salt treated plants

Effect of salinity on grain yield

Different genotypes showed variable reduction in yield characteristics. Most of the *Vigna* species i.e. *V. sublobata*, *V. aconitifolia*, *V. umbellate*, *V. glaberasans* failed to reach reproductive stage due to death of all the plants (0% survival) hence showed 100% yield loss. The genotypes of *V. silvestris* and *V. stipulata*, showed > 80% reduction in grain yield. However, the genotype EC528960 produced significant yield and good quality of produce (bold seeds) under high salinity followed by TCR86 (Fig. 7). It was also observed that salt stress along with other pest and diseases caused 80% to 100% yield loss in the crop plants. Reduced yield in mungbean under salt stress may be due to more flowers shedding, reduced photosynthetic efficiency per day of plant to fill the developing seeds, and shattering of the pods [25, 26].

The investigated *Vigna* genotypes showed considerable differences in response towards salinity stress [27]. The genotypes EC528960 (*Vigna luteola*) and TCR86 (*Vigna trilobata*) showed healthy response as less reduction for the observed traits and significant yield under salt stress depicted their greater resistance, were considered as most salt tolerant compared to all other wild genotype exhibited reverse response and taken as most susceptible towards salinity. The genetically diverse accessions resistant to salt stress within the *Vigna* genotypes could be of considerable practical value for studying the mechanism of salt tolerance and for the provision of genetic resources for salinity breeding program [27]. These days agricultural research has gained importance. Many workers are studying medicinal plants [28, 29] and soyabean [30].

Fig. 3 Salt resistant wild genotype TCR86 *V. trilobata* at early (A & B) and late vegetative stage (C & D) where A & C: control plants; B and D: salt treated plants

Fig 4.1

Fig. 4.2

Fig. 4 Chlorosis and Necrosis symptoms under salinity stress (4.1 and 4.2) where (A) control plants, (B & C) salt treated plants

Table 1 Details of the wild relatives of mungbean screened for salt tolerance

Sr. No.	Accession No.	Wild species	Sr. No.	Accession No.	Wild species	Genetic Resource
1	IC-120992	*Vigna aconitifolia*	12	BBYD-2703	*Vigna sublobata*	NBPGR, New Delhi-12
2	IC-121015	”	13	BBYD-2711	”	”
3	IC-140622	”	14	BB-2722	”	”
4	IC-36114	”	15	----------	*Vigna glaberasans*	”
5	IC-10141	”	16	BB-2723	*Vigna silvestris*	”
6	IC-472257	”	17	BBYD-2707	*Vigna stipulata*	”
7	IC-39713	”	18	BBYD-2712	*Vigna sublobata*	”
8	IC-140678	”	19	BBYD-2700	”	”
9	IC-39633	”	20	EC 528960	*Vigna luteola*	”
10	PLMO-184	”	21	TCR86	*Vigna trilobata*	”
11	----------	*Vigna umbellata*	22	----------	*Vigna sublobata*	”

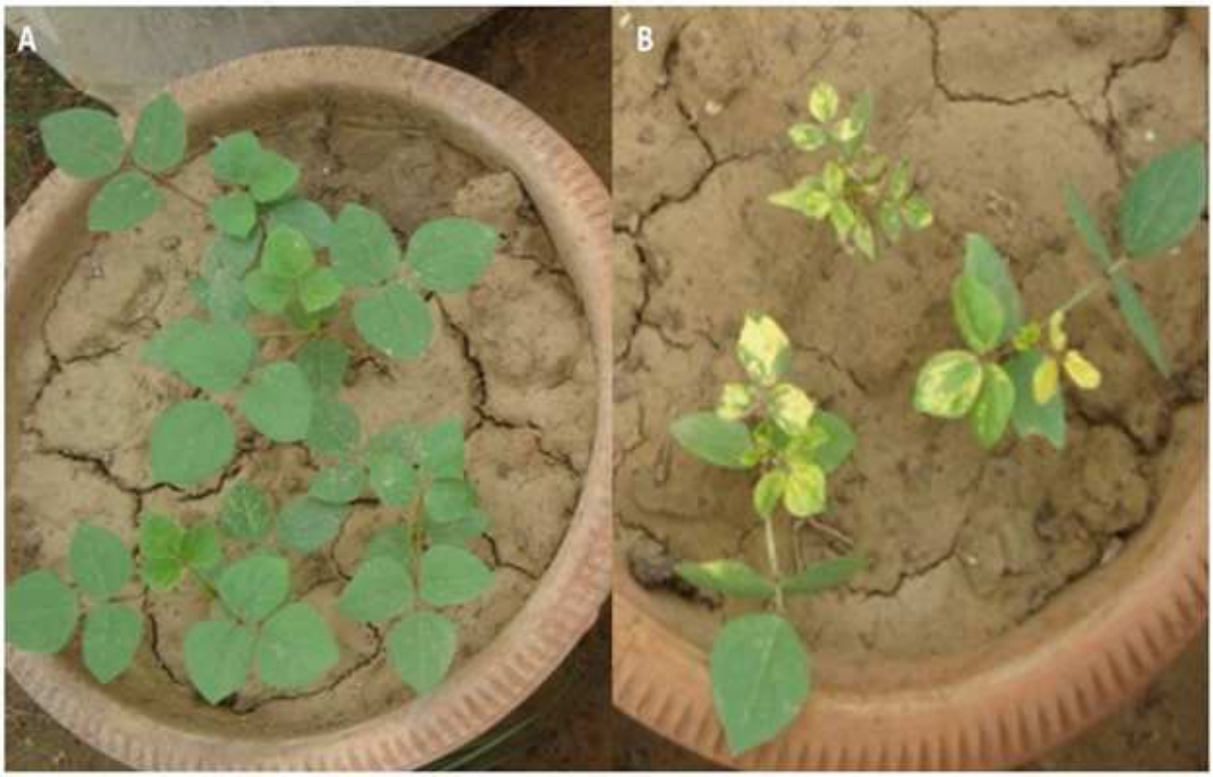

Fig. 5 Yellow mosaic virus symptoms (A) control, (B) salt treated plants

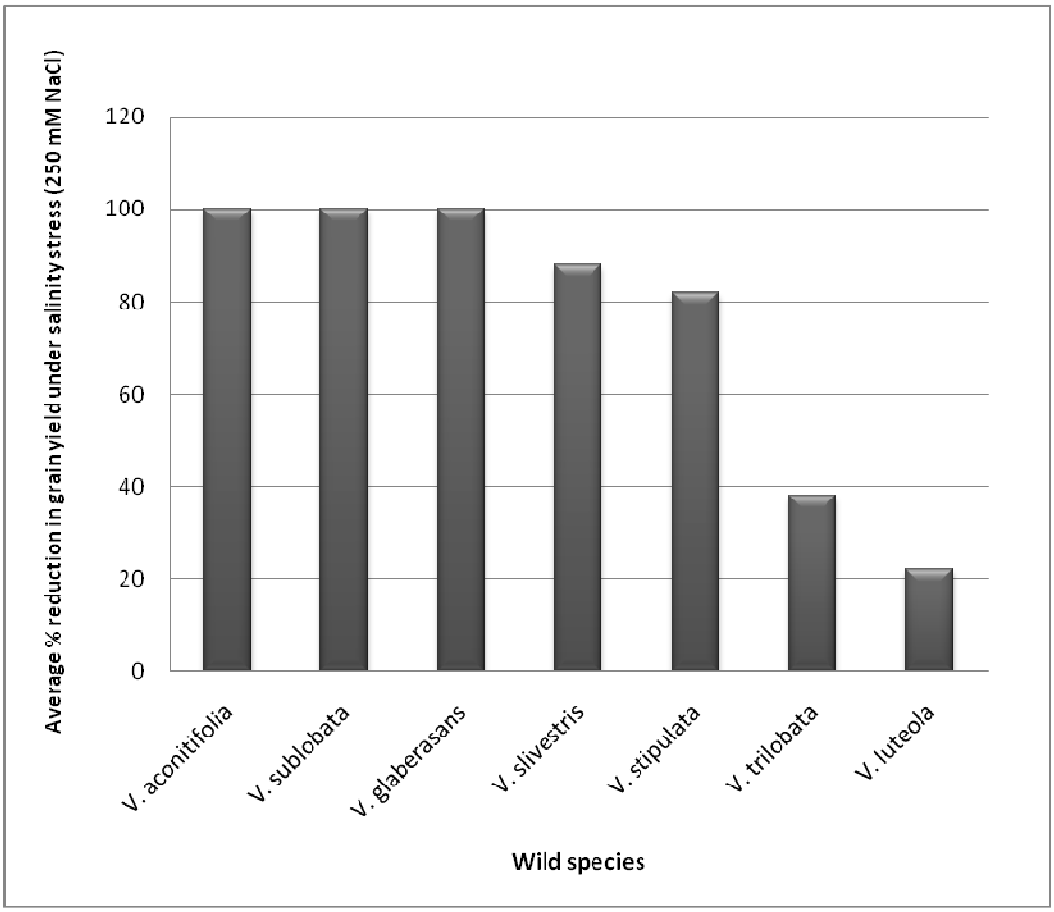

Fig. 6 Average percent reduction obtained in grain yield of different *Vigna* species

CONCLUSION

The study concludes that the genotypes EC528960 and TCR86 could be used as a source of resistance genes to be introgressed in the salt sensitive mungbean genotypes through breeding. The breeding programs should emphasize to involve diverse sources as parental lines for genetic improvement of mungbean for salt tolerance. Development of salt resistant varieties is the most economic and sustainable way to surmount the food paucity of the increasing population world-wide

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