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## Thanniru Bhavyasri

M.Sc. Scholar, Department of Genetics and Plant Breeding, Lovely Professional University, Jalandhar-Delhi G. T Road, Phagwara, Punjab, India

## IR Delvadiya

Assistant Professor, Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Jalandhar-Delhi G. T Road, Phagwara, Punjab, India

## AV Ginoya

Assistant Professor, Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Jalandhar-Delhi G. T Road, Phagwara, Punjab, India

## Corresponding Author:

### Thanniru Bhavyasri

M.Sc. Scholar, Department of Genetics and Plant Breeding, Lovely Professional University, Jalandhar-Delhi G. T Road, Phagwara, Punjab, India

## Breeding of physiological traits and improving the productivity of green gram (*Vigna radiata* L. Wilczek)

Thanniru Bhavyasri, IR Delvadiya and AV Ginoya

### Abstract

Mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*] is an important food and cash legume crop in Asia. The development of short-duration varieties is important for the present climate scenario. Mungbean productivity is constrained by abiotic factors. Key abiotic stresses affecting mungbean production are drought, waterlogging, salinity, and heat stress. It is important to develop varieties with resistance to abiotic factors, but there are many constraints still to address that include the precise and accurate identification of resistance source (s) for some of the traits and the traits conferred by multi genes. Latest technologies in phenotyping, genomics, proteomics, and metabolomics could be of great help to understand insect/ pathogen-plant, plant-environment interactions, and the key components responsible for resistance to biotic and abiotic stresses. This review discusses current abiotic constraints in mungbean production and the challenges in genetic improvement.

**Keywords:** Greengram, climate resilient cultivar, genetic improvement, shorting duration

### Introduction

Greengram is an important protein-rich food legume crop. During the reproductive stage, high temperatures cause flower drop, induce male sterility, impair anthesis, and shortens the grain-filling period, and adaptability of green gram is adversely affected by several abiotic stresses including heat, drought, salinity, and water-logging, which affect crop growth and development by altering physiological processes and the plant-water relationship (Dreesen *et al.*, 2012; Bitra and Gerats, 2013; Suzuki *et al.*, 2014; Kaur *et al.*, 2015; Zandalinas *et al.*, 2017; Landi *et al.*, 2017) [124, 110, 120, 48, 123]. Several studies have reported a reduction in the growth and development of legumes because of high-temperature stress (Tzudir *et al.*, 2014; Hanumantha Rao *et al.*, 2016) [122, 42].

Greengram thrives most effectively at temperatures between 30 °C and 40 °C, however, significant flower shedding occurs at temperatures beyond 40 °C (Zinn *et al.*, 2010; Sita *et al.*, 2017) [124, 119]. Rainey and Griffiths (2005) [118] reported that the abscission of reproductive organs is the primary determinant of yield under heat stress in several grain legumes. The production is considerably influenced by changes in the photoperiod and temperature across the growing regions of greengram extending from low to high latitudes. Because greengram is a quantitative short-day plant (Chauhan and Williams, 2018) [127], short day length at low latitude hastens flower initiation, and the plants rapidly reach the reproductive phase without adequate vegetative biomass production. By contrast, long photoperiod at high latitudes delays the onset of the reproductive phase, but the biomass is adequate and has a high leaf area index.

### Abiotic stresses in mungbean

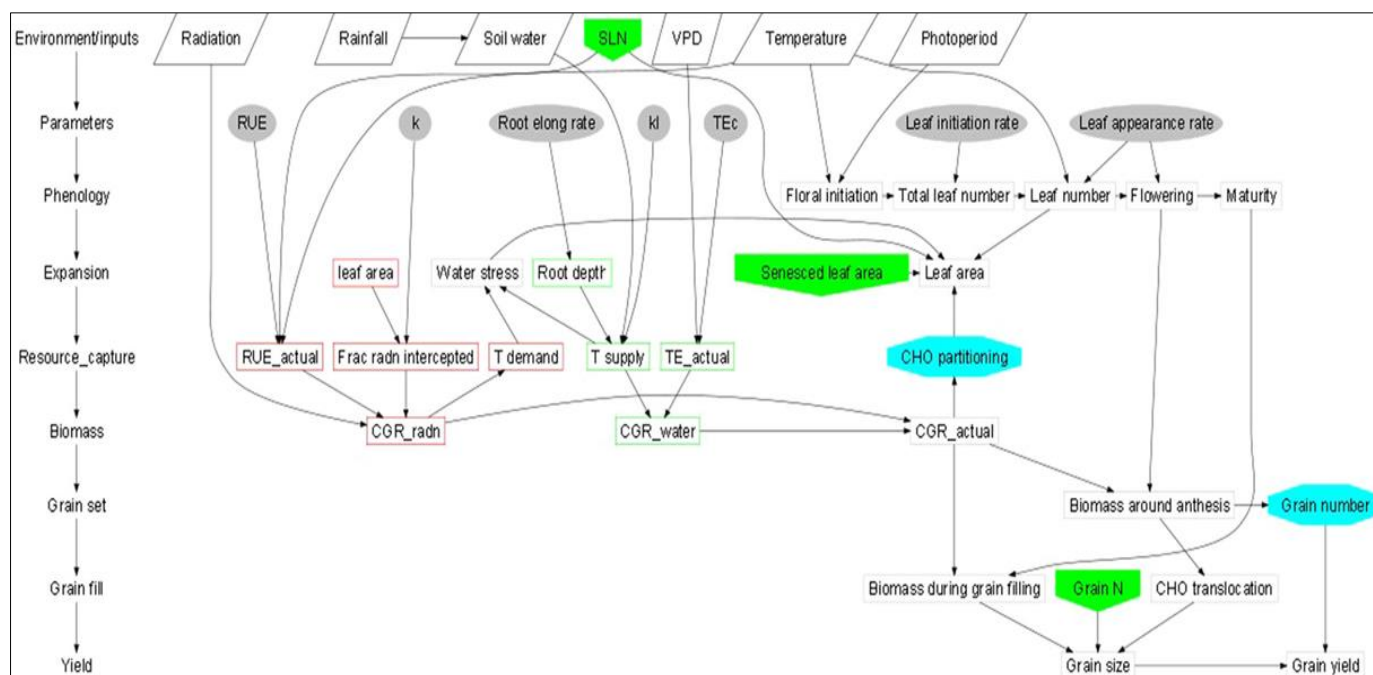
Abiotic stresses negatively influence plant growth and productivity and are the primary cause of extensive agricultural losses worldwide (Arun and Venkateswarlu, 2011; Ye *et al.*, 2017) [13, 105]. Reduction in crop yield due to environment variations has increased steadily over the decades (Boyer *et al.*, 2013) [22]. Abiotic stresses include extreme events and factors related to atmosphere (heat, cold, and frost); water (drought and flooding); radiation (UV and ionizing radiation); soil (salinity, mineral or nutrient deficiency, heavy metal pollutants, pesticide residue, etc.) and mechanical factors (wind, soil compaction) (Hanumantha Rao *et al.*, 2016) [42]. Crops utilize resources (light, water, carbon and mineral nutrients) from their immediate environment for their growth.

The microenvironment and the management practice of cultivation influence crop growth and development directly (Figure 1). Climate change further adds to the complexity of plant-environment interactions (Goyary, 2009) [40]. The eco-physiological models that integrate the understanding of crop physiology and crop responses to environmental cues from detailed phenotyping are therefore used to understand the impact of environmental factors on crop growth and development, predict yield/plant response and also assist in developing management strategies (Figure 2) (APSIM: Chauhan *et al.*, 2010; MungGro: Biswas *et al.*, 2018) [24, 20]. The plant response to abiotic stress at the cellular level is often interconnected (Beck *et al.*, 2007) [15] leading to molecular, biochemical, physiological and morphological changes that affect plant growth, development and productivity (Ahmad and Prasad, 2012) [3]. Several crop production models project a reduction in the crop yields of major agricultural crops mostly due to climate change (Rosenzweig *et al.*, 2014) [79], which tend to make crop growth environment unfavorable due to abiotic stresses. Such efforts in crops like mungbean is rare and requires a special attention. In the current era, environmental stresses are a menace to global agriculture and there is a need to emphasize trait based breeding to ensure yield stability across the locations as well as crop seasons. Efforts are underway to develop new tools for understanding possible mechanisms related to stress tolerance and identification of stress tolerance traits for promoting sustainable agriculture (Cramer *et al.*, 2011; Fiorani and Schurr, 2013) [26, 37]. Basic tolerance mechanisms involve the activation of different stress-regulated genes through integrated cellular as well as molecular responses (Latif *et al.*, 2016) [55]. Plants respond to their immediate surroundings in diverse ways, which assist the cells to adapt and achieve cellular homeostasis manifested in phenotypes of plants under particular environment (James *et al.*, 2011) [47]. While breeding lines are regularly phenotyped for easily visible traits including growth and yield components, many traits that contribute to stress tolerance are ignored. This can be largely due to feasibility of measuring these traits precisely and rapidly. Hence, recent phenotyping tools deploy image capture and automation in advanced plant phenotyping platforms. These recent efforts are expected to boost efforts to translate basic physiology of crop plants into products with practical values to support breeding program in harsh environments (*viz.*, stresses like salinity, soil moisture, extreme temperatures etc) explained in the following section.

### Salinity

In agriculture, soil salinity has been a threat in some parts of the world for over 3000 years (Flowers, 2006) and it has been aggravated by irrigation water sourced through surface irrigation in arid and semi-arid environments (Hanumantha Rao *et al.*, 2016) [42]. Salt stress mainly in most of the crops

reduces seed germination, fresh and dry biomass, shoot and root length, and yield attributes of mungbean (Promila and Kumar, 2000; Rabie, 2005; Ahmed, 2009) [75, 76, 4]. It affects root growth and elongation, thereby, hampering nutrient uptake and distribution. Root growth was significantly reduced with higher Sodium Chloride (NaCl) (NaCl) concentrations. Nevertheless, BARI Mung4 showed better performances at higher NaCl concentration considering a yield-contributing character. Nodules/plant decreased with the increase of salinity although the nodule size increased (Naher and Alam, 2010) [66]. Being polygenic in nature, salinity tolerance is genotype-dependent and growth stage-specific phenomenon, therefore, tolerance at an initial (seedling) stage may not be corroborated with tolerance at later growth (maturity) stages (Sehrawat *et al.*, 2013) [83]. It also involves multidimensional responses at several organ levels in plants (e.g., tissue, molecular, physiological and plant canopy levels) (Hanumantha Rao *et al.*, 2016) [42]. Because of this complexity and lack of appropriate techniques for introgression, little progress has been achieved in developing salt-tolerant mungbean varieties over years (Ambede *et al.*, 2012; Hanumantha Rao *et al.*, 2016) [8, 42]. Appreciable improvement in salt tolerance of important crops (barley, rice, pearl millet, maize, sorghum, alfalfa, and many grass species) have been attained in the past, but not in legumes in general and mungbean to feasibility of measuring these traits precisely and rapidly. Hence, recent phenotyping tools deploy image capture and automation in advanced plant phenotyping platforms. These recent efforts are expected to boost efforts to translate basic physiology of crop plants into products with practical values to support breeding program in harsh environments (*viz.*, stresses like salinity, soil moisture, extreme temperatures etc) explained in the following section. in particular (Ambede *et al.*, 2012) [8]. Rapid screening methods are required to identify putative donor parents in a breeding program (Saha *et al.*, 2010) [81]. In a comprehensive study, Manasa *et al.* (2017) [59] screened 40 mungbean lines sourced from World Vegetable Center for salinity tolerance using Salinity Induction Response (SIR) technique at the seedling as well as at whole plant levels by canopy phenotyping assay under 150 and 300 mM NaCl stress scenario. The results showed a marked reduction in growth and yield performances of both tolerant and susceptible lines, but a few lines displayed a relatively better biomass and pod yield on par with non-stressed control plants. The intrinsic ability of salt partitioning to vacuole (more influx of Na<sup>+</sup> ions) by tolerant lines during high salt concentration in the cytosol could be one of the reasons for their tolerance. Based on the extent of salt tolerance both at seedling and whole plant stages, a few salt tolerant (EC 693357, 58, 66, 71, and ML1299) lines were identified (Manasa *et al.*, 2017) [59] for further validation under field conditions.



**Fig 1:** Schematic representations of crop growth and development dynamics

### Soil moisture stress

The response of legumes to the onset of drought vary and the final harvestable yield will significantly be reduced (Nadeem *et al.*, 2019) [65]. Global climate change attributes erratic prediction in drought episodes and its control of crop yields. Being grown on marginal lands, mungbean is largely considered as a drought tolerant (grow with a limited soil moisture). However, like any other plants, it responds to a decrease in available soil moisture by reducing its growth and hence productivity. It is evident from the experiment that 30% decrease in water supply relative to water optimum for crop growth results in nearly 20% decrease in seed weight per plant if the soil moisture stress imposed around a vegetative stage. The plants subjected to stress during flowering showed 50 to 60% decrease in seed yield (Fathy *et al.*, 2018) [33]. Soil moisture stress did not affect the number of pods per plant as severely as it did for seed weight or biomass per plant in this experiment, clearly indicating that seed formation or filling is the most sensitive to soil moisture stress.

It is also suggested that dry matter partitioning is one of the potential screening traits for drought tolerance in mungbean (Hossain *et al.*, 2010; Nadeem *et al.*, 2019) [44, 65] at the cellular level. Hence, key factors that can alleviate oxidative stress are the focus of research for alleviating drought stress. Recent studies infer that alleviation of drought-caused oxidative stress depends largely on the status of Ascorbic acid and Glutathione pools in reduced and oxidative stages (Anjum *et al.*, 2015) [11]. There is a need to explore genetic variation for these traits and possibility of introgressing the relevant genes for improving drought tolerance in mungbean.

Decreased leaf water potential was associated with reduced activity of nitrogenase, glutamine synthetase, asparagine synthetase, aspartate aminotransferase, xanthine dehydrogenase, and uricase which are associated with nitrogen fixation (Kaur *et al.*, 1985) [48]. New insights into these metabolites and enzymes can be obtained to understand their roles through recently evolved metabolomics.

Water stress-induced inhibition of hypocotyl elongation is more conspicuous in separated cotyledons than the intact ones. It is necessary to check if the larger cotyledons can be the solution for better plant establishment under soil moisture stress. When two mungbean genotypes exhibiting more than two-fold variation in leaf water loss were explored for the genetic variation in their physiological and molecular responses to drought, efficient stomatal regulation was observed in water saving low leaf water loss (LWL) genotype (Raina *et al.*, 2016) [77]. The stomatal closure under drought was accompanied with a concomitant down-regulation of farnesyl transferase gene in this genotype. However, other genotypes had a cooler canopy temperature facilitated by a branched root system that allowed better extraction of soil moisture (Raina *et al.*, 2016) [77]. These mechanisms and traits of mungbean are suitable for harsh environments but needs a prioritization based on the type of drought and agro-ecological features. The other important key physiological traits *viz.*, water use efficiency, root growth/biomass, carbon isotope discrimination ( $\Delta^{13}C$ ) and leaf temperature (Canopy temperature difference), may be beneficial for screening mungbean for drought tolerance

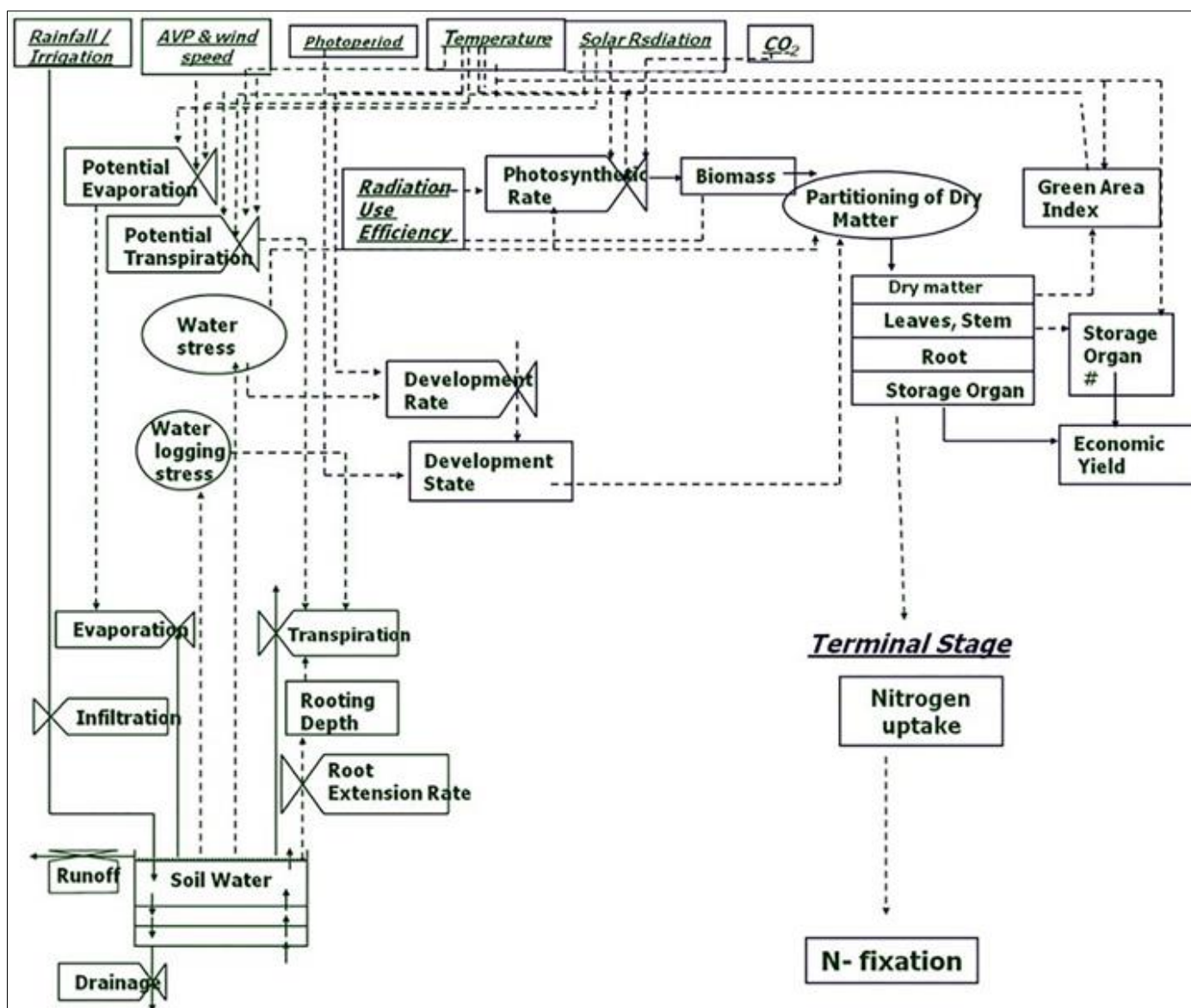


Fig 2: Process chart of Mungbean growth model

### High temperature or heat stress and increasing atmospheric carbon dioxide (CO<sub>2</sub>)

Of the various environmental stresses that a plant can experience, temperature has the widest and far-reaching effects on legumes. Temperature extremes, both high (heat stress) and low (cold stress), are injurious to plants at all stages of development, resulting in severe loss of productivity. Legumes, such as chickpea, lentil, mungbean, soybean, and peas, show varying degrees of sensitivity to high and low-temperature stresses, which reduces their potential performance at different developmental stages such as germination, seedling emergence, vegetative phase, flowering, and pod/seed filling phase (Hanumantha Rao *et al.*, 2016; Sharma *et al.*, 2016) <sup>[42, 89]</sup>. The optimum temperature for growth and development of mungbean is 28–30 °C and the range under which plant continues to develop seed is 33–35°C. Each degree rise in temperatures above optimum reduces the seed yield by 35–40% relative to the plants grown under optimum temperature (Sharma *et al.*, 2016) <sup>[89]</sup>. Temperatures >45 °C that often coincides at flowering stage can lead to flower abortion and yield losses. Sharma *et al.* (2016) <sup>[89]</sup> evaluated the effect of high temperature on different mungbean lines for vegetative and reproductive

performances using Temperature Induction Response (TIR) and physiological screening, techniques at seedling and whole plant levels. The promising tolerant lines were shortlisted for further investigation at the whole plant level. These lines were grown in containers under full irrigation in outdoors; screened for growth and yield traits at two sowings: normal sowing (NS), where day/night temperatures during reproductive stage were <40/28 °C, and late sowing (LS), where temperatures were higher (> 40/28 °C). The leaves of LS plants showed symptoms of leaf rolling and chlorosis and accelerated phenology lead to sizable marked reduction in leaf area, biomass, flowers and pods. Interestingly, shortening of flowering and podding duration was also observed.

To address ever-fluctuating temperature extremes that various legumes get exposed to, efforts are being made to develop heat-tolerant varieties through conventional breeding methods (exposing breeding lines to open air growing seasons having high temperature episodes either throughout the growth stages or specific to flowering or reproductive phase) in order to select promising tolerant lines. Subsequently subject these shortlisted entries to varied growing environments that coincide with drier/heat periods for confirmatory validation to identify true-genotypes to engage

them in heat stress breeding programs. With the advancement of 'omics' era, phenomics platform (phenotyping) can conveniently be applied to screen field shortlisted or promising sub-set of candidates with more precisely conditioned high-temperature regimes (at customized growth periods) to identify true types along with expressed plant architectures. Tolerance to suboptimal temperatures has not been studied extensively in crops like mungbean. However, for the improvement in grain yield of this crop in hilly areas or in higher latitudes it is necessary to introgress traits associated with cold or low-temperature tolerance.

Increasing atmospheric CO<sub>2</sub> concentration along with temperature also pose a constraint to plant growth and development, which would be more pronounced in C<sub>3</sub> plant species (like mungbean) than C<sub>4</sub>. Some of the physiological functions (activation of carboxylating enzymes, photosynthetic rates, cell expansion, carbohydrate synthesis etc) will be enhanced which have an impact on leaf area and biomass associated improvements. An improved biomass by virtue of increased leaf expansion may not always result in higher yield levels. However, in mungbean, higher pod and seed yields were documented when a few high temperature tolerant genotypes exposed to elevated CO<sub>2</sub> of 550 ppm compared to ambient CO<sub>2</sub> of 400 ppm (Bindumadhava *et al.*, 2018) [19]. However, molecular mechanism governing aggravated metabolic functions at different growth stages is still unclear and possibility of employing CO<sub>2</sub> fertigation as a breed able trait needs more research attention in days to come from the context of changing global climate.

### Waterlogging

Anthropogenic studies reveal that the frequency and severity of flooding events increase with climate change (Arnell and Liu, 2001) [12]. Waterlogging adversely affects germination, seedling emergence and growth, crop establishment and root and shoot growth (Bailey- Serres and Voeselek, 2008; Toker and Mutlu, 2011) [14, 96]. Heavy rains during pod ripening stage results in premature sprouting, leading to inferior seeds. Mungbean is predominantly cultivated in rice-fallow systems and is sensitive to waterlogging (Singh and Singh, 2011) [91]. Excess rainfall in such cultivation systems can result in waterlogging wherein roots are completely immersed in water and shoots (sometimes) are partially or fully submerged. Ahmed *et al.* (2013) [5] highlighted the biochemical mechanisms *viz.*, increased availability of soluble sugar, enhanced enzymatic activity of glycolytic pathway antioxidant defense mechanism, and altered aerenchyma formation help plants withstand waterlogging. In addition to the deficiency of oxygen, waterlogging can alter the mineral nutrient composition accessible for plants and needs to be considered during genetic crop improvement (Setter *et al.*, 2009) [86]. Spring grown crops are more prone to water stress as the rainfall is scanty and farmers mostly prefer to grow this crop on residual moisture. Therefore, cultivating short duration cultivars may help in escaping terminal moisture stress (Pratap *et al.*, 2013) [72].

### Breeding for abiotic traits

At the plant level, there were several satisfying attempts in mungbean to screen and identify tolerant types for high temperature (heat stress), salinity, waterlogging, and water stress from physiological, biochemical, and molecular perspectives (Kaur *et al.*, 2015; Hanumantha Rao *et al.*, 2016; Bhandari *et al.*, 2017; Manasa *et al.*, 2017; Sehgal *et al.*, 2018) [48, 42, 17, 59, 82]. The breeding lines selected and identified for these aforementioned stresses would form a panel of donor resources for future trait-navigated crop improvement (Table 1).

The initial phase of breeding in mungbean resulted in selecting a few locally adapted germplasm, mainly for biotic stresses resistance and high yield. While selecting for abiotic stress resistance was not practiced directly, selection for yield, plant type, and adaptation related traits indirectly lead to selection for abiotic stress resistance as well. The selection has been a useful strategy to identify superior cultivars with significant drought tolerance. Warm season food legumes generally encounter two types of drought stresses: (i) terminal drought, which is more prominent in summer/spring crops, usually coincides with late reproductive stage and increases towards generative stage, and (ii) intermittent drought, which may occur anytime during vegetative growth and results due to a break in rainfall or insufficient rains at the vegetative stage. The ranking of warm season food legumes in increasing order of drought resistance was soybean, followed by blackgram, mungbean, groundnut, bambara nut, lablab bean and cowpea (Singh *et al.*, 1999) [92]. Fernandez and Kuo (1993) [34] used a stress tolerance index (STI) to select genotypes with high yield and tolerance to temperature and water stresses in mungbean. Singh (1997) [90] described the plant type of mungbean suitable for Kharif (rainy) as well as dry (spring/summer) seasons. Pratap *et al.* (2013) [72] also suggested the development of short duration cultivars for Spring/Summer cultivation so that these escape terminal heat and drought stress. Cultivars with 60–65 days' crop cycle, determinate growth habit, high harvest index, reduced photoperiod sensitivity, fast initial growth, longer pods with more than 10 seeds/pod and large seeds are more suitable to the summer season. Keeping this backdrop, a number of early maturing mungbean lines have been selected and released as commercial cultivars larvae and larval mortality (Xiong *et al.*, 2013) [125]. Additionally, this technology has been implicated in increasing the production of unique secondary metabolites, increasing the shelf life of the fruits, improving crop yield and improving and disease resistance (Abhary and Rezk, 2015) [1]. Sunkar and Zhu (2004) [126] reported that in *Arabidopsis* plants, miRNAs are involved in tolerance against abiotic stress including cold, drought, and salinity. They further showed that exposure to higher salinity levels, dehydration, cold, and abscisic acid upregulated the expression of miR393. While RNAi technology can be used to improve biotic and abiotic stress resistance/tolerance in mungbean, large-scale field studies are needed to study any potential risks of this technology.

**Table 1:** Tolerant/resistant sources of mungbean against abiotic stresses.

Abiotic stress	Source of tolerance	Country	Reference
Drought	K-851	India	Dutta and Bera (2008), Dutta <i>et al.</i> (2016) <sup>[31, 30]</sup>
Heat tolerance and elevated CO <sub>2</sub> levels	EC693357, EC693358, EC693369, Harsha and ML1299	India	Sharma <i>et al.</i> (2016), Bindumadhava <i>et al.</i> (2018) <sup>[89, 19]</sup>
Drought	TCR 20	India	Tripathy <i>et al.</i> (2016) <sup>[97]</sup>
Drought	SML-1411, SML-1136	India	Kaur <i>et al.</i> (2017) <sup>[49]</sup>
Drought	ML 267	India	Swathi <i>et al.</i> (2017) <sup>[93]</sup>
Drought	VC 2917 (seedling stage)	China	Wang <i>et al.</i> (2014, 2015) <sup>[100, 99]</sup>
Drought	V-1281, V-2013 and V-3372	Taiwan	AVRDC (1979) <sup>[109]</sup>
Waterlogging	V 1968, V 2984, V 3092 and V 3372	Taiwan	AVRDC (1979) <sup>[109]</sup>
Drought	VC 1163 D, VC 2570A, VC 2754 A and VC 2768 A	Taiwan	Fernandez and Shanmugasundaram (1988) <sup>[35]</sup>
Drought & Flooding	V 1381 and VC 2778	China	He <i>et al.</i> (1988) <sup>[128]</sup>
Low temperature	Perennial accessions of <i>V. radiata</i> var. <i>sublobata</i>	Taiwan	Lawn <i>et al.</i> (1988) <sup>[56]</sup>
Salt	S72, H45, No. 525, Madira and RS-4	India	Maliwal and Paliwal (1982) <sup>[58]</sup>
Salt	T-44	India	Misra and Gupta (2006) <sup>[62]</sup>
Salt	BARI Mung-4	Bangladesh	Naher and Alam (2010) <sup>[66]</sup>
Salt	NM 19-19	Pakistan	Shakeel and Mansoor (2012) <sup>[87]</sup>
Salt	TCR86, PLM380, PLM562, WGG37, IC615, PLM891, IC2056, IC10492, PLM32, K851, and BB92R	India	Shrawat <i>et al.</i> (2014) <sup>[84]</sup>
Salt	EC 693357, 58, 66, 71 and ML 1299	India	Manasa <i>et al.</i> (2017) <sup>[59]</sup>
Pre-harvest sprouting	Chamu 4	India	Lamichaney <i>et al.</i> (2017) <sup>[53]</sup>
Heat	IPM 02-16, IPM 9901-10, IPM 409-4, IPM 02-3, PDM 139, IPM 02-1, IPM 2-14, IPM 9-43-K, PDM 288, EC 470096, IPM 2K14-9, IPM 2K14-5	India	Khattak <i>et al.</i> (2009) <sup>[50]</sup>
Drought (maintaining cooler canopy traits)	VC-6173-C, IC-325770, ML 2082	India	Raina <i>et al.</i> (2016) <sup>[77]</sup>

### Breeding constraints for developing abiotic stress resistant/tolerant mungbean

In breeding for resistance to abiotic stresses in legumes, the important factors that are taken into consideration include the genetic distance between the resistant source and the cultivars to be improved, screening methodology, inheritance pattern and the resistance traits to be improved. The genetic diversity and the genetic distances between cultivars and the resistance sources can be integrated in breeding approach such as gene pyramiding (Kelly *et al.*, 1998; Kim *et al.*, 2015) <sup>[114, 51]</sup>. The important breeding approaches such as the pedigree and single seed descent methods are used to transfer the major resistant alleles and QTLs between cultivars and elite breeding lines. However, the increased genetic distances between the source and the cultivars lead to segregation of characters, which can be reduced by repeated backcrossing such as inbred-backcrossing, recurrent backcrossing, or congruity backcrossing (i.e., backcrossing alternately with either parent). During early stages of the breeding program for breeding to diseases and resistance, introgressing resistance alleles and QTL from wild populations, recurrent or congruity backcrossing or modifications are highly important. Although gamete selection using multiple-parent crosses (Asensio-S.-Manzanera *et al.*, 2005, Asensio-S.-Manzanera *et al.*, 2006) <sup>[108, 107]</sup> and recurrent selection (Kelly and Adams, 1987; Singh *et al.*, 1999; Terán and Singh, 2010) <sup>[114, 94, 121]</sup>, respectively, could be effective, their use in the legumes where a large number of pollinations are required may not be feasible. Linkage drag is one of the important challenges while developing the disease or resistant cultivars, especially when wild sources are used as donors. To reduce linkage drag, repeated backcrossings are needed (Kenenen *et al.*, 2011) <sup>[115]</sup>. Deployment of wild germplasm in resistance breeding, which is an important source of resistance introgression to commercial cultivars, is often impeded by the undesirable genetic linkages, which may result in the co-inheritance of the

undesired and desired traits that may affect seed quality, germination and other traits (Edwards and Singh, 2006; Acosta-Gallegos *et al.*, 2008; Kenen *et al.*, 2011) <sup>[32, 2, 115]</sup>. Gene pyramiding the incorporation of multiple resistant genes in a cultivar is seen as an alternative to breeding for diseases/resistance with several strains/biotypes.

Though there have been several continued attempts to evolve crop varieties/genotypes for a specific biotic and abiotic stress, on a larger scale, the success achieved was less owing to the combined impact of several stresses and unexpected sudden episodes of pests and diseases all along growth stages of the plants; hence, only a few countable successes have been reported in legumes, more so in cereals. Stemming the critical stage of crop growth for breeding itself need a thorough assessment, be seed germination, early vigour or field establishment, vegetative phase, flowering and early podding to podding stage, reproductive to final maturity stages etc. In this array of developmental stages, pinning down a specific stage and the very influencing trait for breeding seems very challenging though several strategies have hovered around flowering and reproductive phase (being termed 'sensitive') with an objective to develop breeding lines that withstand stress load and produce relatively better pod and seed yield.

### Conclusion

Molecular approaches are becoming handy in revealing resistance/tolerance mechanisms, which will help in modifying mungbean plants to suit the abiotic stresses. Genome Wide Association Studies (Noble *et al.*, 2018; Breria *et al.*, 2019) <sup>[117, 111]</sup> would help in better understanding of the genetic basis of the phenotypes. Association mapping for abiotic resistant/ tolerant traits is highly important to identify the desired haplotypes in performing association mapping on a panel of adapted elite breeding lines. This will provide the ample justification to utilize these lines directly in breeding

programs. The selection of favorable haplotypes through MAS will be reduce the phenotyping material in the advanced breeding generations and increase the breeding efficiency.

### Future outlook

Breeding mungbean lines for stressful environments is very important. While in particular, stress dominates a population of environments, many of the agroecologies are featured by multiple stresses. This often makes a particular agro-ecology unique for which systemized solutions are essential. For making the best combination of abiotic stress and the traits to incorporate, it is essential to have insight on the fundamental mechanism for stress tolerance from intrinsic physiological and biochemical perspectives. We aim to develop root systems that help plants to withstand moisture deficits by drawing water from the deeper soils. Screening for various abiotic stresses needs to be more precise and stringent to identify robust donor/s for these traits. The identified donors need to put in use by the breeders at a faster pace. Plant type/s having a deep root system, early maturity span, erect stature with sympodial pod-bearing, multiple pods per cluster and longer pods with many nodes and shorter internodes will help in withstanding heat and drought-related stresses. Of late, converging various modern technologies like, infra-red thermography, automated robotics, camera images, and computational algorithms, which all make components of high throughput phenotyping facilities (phenomics and phenospex) can facilitate high throughput phenotyping for stress tolerance (Pratap *et al.*, 2019b)<sup>[74]</sup>. However, non-destructive methods being utilized for targeted regions or environments needs optimization for establishing a relation between the known difficult to measure traits and the surrogate parameters derived from images, which represent plant responses to abiotic stresses. These phenomics methods can help precisely quantifying plant shoot architectural responses to stresses caused by soil moisture deficit, salinity, high temperature etc. More than a dozen image parameters have been explained to illustrate the responses of plants to stress that can guide in identifying the relevant traits and the protocol for screening large number of breeding lines or mapping population that are aiming at identification of stress tolerant genes. As evident from published literature, some of the traits such as high photosynthesis or quantum yields have been associated with tolerance to drought, salinity or high temperature. Generally, it is attributed to the capacity of plants to maintain water balance in the tissue reflected by relative water content and stress avoidance mechanism. However, it is essential to look into the traits such as capacity to retain physiological function, for example, even at 50% of optimum relative water content. Such traits are not feasible for application in plant breeding program with conventional approach. However, plant phenomics platform allow no destructive measurement of physiological function such as chlorophyll fluorescence based PS-II system. They are also equipped with NIR-based tools to assess non-destructively tissue water status in plants subjected to stress. These tools can allow measurement of tolerance of PS-II system health at given levels of tissue water content and hence true tolerance to stresses such as soil moisture deficit, salinity and high temperatures. Further, mechanisms to escape from abiotic stresses like drought and high temperatures are extensively been explored in many crops to get optimum yield in stress prone agroecologies. However, there is scope for exploring

diurnal escape from stress in a way that plant can exhibit water saving mechanisms during peak stress hours in the diurnal cycle and keep their stomata open for sufficiently capture ambient CO<sub>2</sub>. It is possible to quantify such traits by strategically employing phenomics tools such as infrared imaging system. High temperatures during nights, is likely to enhance respiratory loss of assimilates, however, there are no mechanisms to measure these traits. It is essential to device tools/ protocols for these measurements either in high or semi-throughput modes. Since mungbean is grown largely in marginal environments or in a short time between harvest and sowing of preceding and subsequent crops, it is essential to assess recovery from stress and performance in terms of seed yield. Continuous monitoring image based system can allow precise quantification of these traits by separating developmental changes from actual impact of stress. Recently evolved CT scanbased tools and protocols will allow understand root-soil-water interaction and can quantify roots system architecture more precisely. This will open up new avenues for designing phenomics and genomics approaches for supporting improvement of stress tolerance in crops.

Molecular approaches are becoming handy in revealing resistance/tolerance mechanisms, which will help in modifying mungbean plants to suit the abiotic stresses. Genome Wide Association Studies (Noble *et al.*, 2018; Breria *et al.*, 2019)<sup>[117, 111]</sup> would help in better understanding of the genetic basis of the phenotypes. Association mapping for abiotic resistant/ tolerant traits is highly important to identify the desired haplotypes in performing association mapping on a panel of adapted elite breeding lines. This will provide the ample justification to utilize these lines directly in breeding programs. The selection of favorable haplotypes through MAS will be reduce the phenotyping material in the advanced breeding generations and increase the breeding efficiency. The development of NGS technologies, the discovery of SNP/alleles has become easy. This mungbean diversity panel constitutes a valuable resource for genetic dissection of important agronomic traits to accelerate mungbean breeding. Genetic variability with mungbean and between closely related species can be studied from the sequence-based information, which forms a pre-requisite criterion for breeding for resistant/tolerance to biotic and abiotic stress. This is also important for the species conservation and provides breeders with new and/ or beneficial alleles for developing advanced breeding materials. Further, advanced phenotyping technologies such as NGS help to increase the discovery of trait-allele and genotype-phenotype interactions. There must be systematic efforts towards exploring physiological and biochemical regulations of biotic and abiotic stresses and studying the whole profile of genes, proteins and metabolites imparting resistance/tolerance so that the same can be manipulated to develop improved cultivars of Mungbean.

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