# A Review on Stress Physiology and Breeding Potential of an Underutilized, Multipurpose Legume: Rice Bean (*Vigna umbellata*)

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## Abstract

Pulses and legumes are reliable dietary supplements for people of the developing countries. Ricebean [*Vigna umbellata (Thunb.) Ohwi and Ohashi*] is an underutilized, multipurpose legume owing to its profuse pod-bearing habit, wider adaptability, tolerance to biotic and abiotic stresses, high seed yield, and high contents of nutrients considered as a minor food and fodder crop. It is a potential leguminous crop with luxuriant growth habits and ability to produce huge quantity of nutritive green fodder as well as high seed yield under limited management inputs. Ricebean has source of high-quality proteins, calcium, phosphorus, tryptophan, and starch in its seeds. It is mostly cultivated for human consumption; however, its foliage and dry straw are nutritious livestock feed and provide higher productivity and quality fodder if sown with cereals like maize and sorghum. Ricebean thrives well in rainfed areas and also poor soils owing to its wider adaptability and resilience to various abiotic stresses. In view of the climate change scenario, ricebean has enormous potential as a climate-smart legume crop for areas having drought or salinity stress, etc. In this article, an attempt has been made to discuss aspects and scope of abiotic stress-tolerant ricebean genotypes.

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## 11.1 Introduction

Ricebean [*Vigna umbellata (Thunb) Ohwi and Ohashi*] is an ancient leguminous crop of South, Southeast, and East Asia (Seehalak et al. 2006; Tomooka et al. 2002; Pattanayak et al. 2019), which is mainly grown by subsistence farmers for its ability to produce a huge quantity of nutritive green fodder under limited management inputs (Chatterjee and Dana 1977; Chatterjee and Mukherjee 1979). It is also called as "under-utilized" legume or "orphan" crops or poor man's pulse. Legumes such as mungbean, chickpea, pigeon pea, and lentils are widely cultivated in India and abroad. But the poor availability of soils and various biotic and abiotic stresses have reduced the production and productivity of the crops (Sultana et al. 2014). Farmers and consumers need a cost-effective, stress-tolerant alternative crop for their increasing demands. Crop diversification provides better opportunities for creating a uniform cropping system as well as developing resistance against biotic and abiotic stress. Underutilized legumes play an important role in such scenario providing better alternatives with high degrees of stress tolerance. One such underutilized legume is rice bean (*Vigna umbellata*) which has great potential as an alternative with high nutritional and economic value (Dhillon and Tanwar 2018). It has recently gained attention as an underutilized grain legume owing to its profuse pod-bearing habit, wider adaptability, tolerance to biotic and abiotic stresses, high seed yield, and high contents of protein, calcium, phosphorus, tryptophan as well as starch in its seeds (Singh et al. 1980; Srivastava et al. 2001). It performs well in marginal lands, rainfed areas, drought-prone areas, and exhausted soils. It is a neglected crop under diverse conditions with no additional inputs, cultivated on small areas by subsistence farmers in hill areas of Nepal, northern and northeastern India, and parts of southeast Asia.

Continuous supply of nutrients, biosynthetic capacity, and energy are required for proper cell division and normal plant growth. Restriction of any of these most important factors may cease plant growth which leads to plant death. Deviation to normal physiology, growth, developmental processes, and metabolic functions that lead to an injurious effect as well as irreversible damage to the plants can be termed stress (Pareek et al. 2009). Biotic stresses include many living organisms such as fungi, bacteria, virus, insect, and nematode, which draw nutrition parasitizing the host plants. On the other hand, non-living things on which plants grow and develop strongly depend may contribute to abiotic stresses when their supply is irrational. Modern agriculture faces many biotic and abiotic stresses that challenge crop cultivation to a great extent. Several reports highlight massive loss in crop production under environmental stresses (Godfray et al. 2010; Cramer et al. 2011).

Inadequate supply of soil water, relative humidity, excess soil salinity, acidity or basicity, heavy metals, and physiochemical properties may cause stress on plants.

Therefore, the improvement of existing commercial varieties and other plant species to ensure their survival coping with various stresses is the major thrust of plant research. Proper anticipation of physiological and molecular changes under adverse environments to bolster an effective acclamatory response is quite necessary to adopt suitable breeding strategies. Abiotic stresses and their impact on plant performances may be explored in a great variety of model and crop species from different aspects: metabolic or physiological response, signaling pathway, eco-physiology, and crop breeding studies (Pareek et al. 2009).

## 11.2 "Ricebean: A General Overview"

The center of origin and diversity of ricebean is considered to be Indo-China (Tamooka et al. 1991) mainly native of South and South East Asia. It is cultivated in India, Burma, Malaysia, China, Korea, Indonesia, Philippines, and to a limited extent in West Indies, Australia, United States, and East Africa. In India, it is predominantly grown in tribal regions of North-eastern hills, eastern peninsula tracts of Orissa, and chotanagpur, and in hilly regions of North Bengal and Sikkim. It is known by different local names in different parts of the country, such as Masyang, Jhilinge, Gurous, or Siltung. There are many landraces of ricebean under cultivation in different parts of the country. These landraces are mostly low yielding. Plant breeding efforts for genetic improvement and the development of new varieties of ricebean have not yet been carried out successfully (Khadka and Acharya 2009).

It is believed that ricebean evolved from its wild form, *Vigna umbellata var. gracilis*, which is an indeterminate growth habit with photoperiod sensitive, sporadic and asynchronous flowering, typically small leaved, freely branching, and strongly dehiscent pods with small and hard seeds. Ricebean is understood for its diverse distribution and range of adaptation from humid subtropical to warm and cool temperate climate. Ricebean is distributed from southern China through the north of Vietnam, Laos, and Thailand to Burma, India, and Nepal. Its successful cultivation in Queensland and East Africa has also been reported. However, there are reports that ricebean is also cultivated commonly in Honduras, Brazil, and Mexico too.

### 11.2.1 Diversity of Ricebean

Ricebean is an annual legume with an erect to semi-erect vine that may grow to more than 3 m height. It produces profuse branching. Leaves are tri-foliate, leaflets being comparatively broader and hairy. Flowers are conspicuously bright yellow and borne in clusters. Flowering is asynchronous, and there is a tendency to hard seeds. In many areas, landraces that retain many of these characteristics persist, in particular with regard to daylight sensitivity, growth habits, and hard seeds. Ricebean is a

**Table 11.1** Different characteristics of landraces

| Sl. No. | Landraces | Different characteristics |
|---|---|---|
| 1 | Early small grained | 1. Greenish to yellowish in color<br>2. Early in maturity<br>3. Small-sized grain and<br>4. Low grain and fodder |
| 2 | White big grained | 1. Big sized grain,<br>2. White to yellowish in color<br>3. Late in maturity<br>4. Better in taste<br>5. High grain and fodder yield. |
| 3 | Red grained | 1. Small to medium-sized grain<br>2. Medium in maturity<br>3. Red in color<br>4. Least affected by rainfall<br>5. Coarse grain<br>6. Low grain yield |
| 4 | Big brown grained | 1. Brown striped in color<br>2. Big sized grained<br>3. Late in maturity<br>4. Better taste |

Source: LI-BIRD (2007)

diploid ($2n = 22$), and there is some evidence of natural out-crossing (Sastrapradja and Sutarno 1977). It has elongated, slightly curved, and beaked seeds of variable size and color with predominant hilum. In Nepal, there are very few scientific studies conducted on ricebean, and little has been done to assess its diversity and to promote it as a livelihood supporting grain legume.

Ricebean is a multipurpose crop, mainly used for human dietary uptake, with a smaller proportion used for fodder and green manuring (Joshi et al. 2006). It forms an important part of cereal-based diet as the dried grains are rich in protein, minerals, and vitamins. Besides, it carries social and cultural values in some communities in the country. The promotion of ricebean as a crop could play a vital role in improving the human diet and food security of people (Table 11.1).

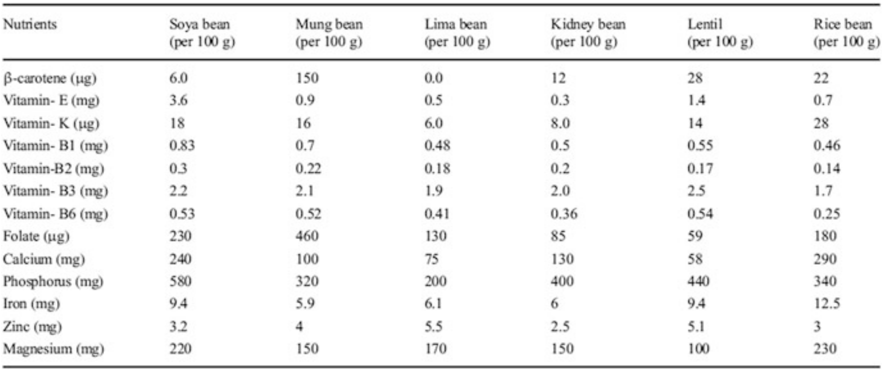
### 11.2.2 Nutritional Factors

In the human diet, legumes are an excellent source of macronutrients, not only carbohydrates but particularly protein as well as healthy fats and dietary fiber. They are also a good source of micronutrients and vitamins. Ricebean is highly nutritious. In terms of nutritionary worth, rice bean is proportional supplementary low-fat grain pulses. The dry seeds of ricebean are good sources of carbohydrates, proteins, minerals, and vitamins. Protein content in ricebean is rich in limiting amino acids tryptophan and methionine (De Carvalho and Vieira 1996). Other amino acids including tyrosine, valine, and lysine (Mohan and Janardhan 1994) are very high in the seeds of ricebean (Table 11.2).

**Table 11.2** Biochemical constituents of ricebean

| Constituent | Range (%) |
|---|---|
| Carbohydrate | 58.2–72.0 |
| Crude protein | 18.3–32.2 |
| Ash | 3.5–4.9 |
| Soluble ether extract | 0.1–0.5 |
| Crude fiber | 3.6–5.5 |

Source: Buergelt et al. (2009)



| Nutrients | Soya bean (per 100 g) | Mung bean (per 100 g) | Lima bean (per 100 g) | Kidney bean (per 100 g) | Lentil (per 100 g) | Rice bean (per 100 g) |
|---|---|---|---|---|---|---|
| β-carotene (μg) | 6.0 | 150 | 0.0 | 12 | 28 | 22 |
| Vitamin- E (mg) | 3.6 | 0.9 | 0.5 | 0.3 | 1.4 | 0.7 |
| Vitamin- K (μg) | 18 | 16 | 6.0 | 8.0 | 14 | 28 |
| Vitamin- B1 (mg) | 0.83 | 0.7 | 0.48 | 0.5 | 0.55 | 0.46 |
| Vitamin-B2 (mg) | 0.3 | 0.22 | 0.18 | 0.2 | 0.17 | 0.14 |
| Vitamin- B3 (mg) | 2.2 | 2.1 | 1.9 | 2.0 | 2.5 | 1.7 |
| Vitamin- B6 (mg) | 0.53 | 0.52 | 0.41 | 0.36 | 0.54 | 0.25 |
| Folate (μg) | 230 | 460 | 130 | 85 | 59 | 180 |
| Calcium (mg) | 240 | 100 | 75 | 130 | 58 | 290 |
| Phosphorus (mg) | 580 | 320 | 200 | 400 | 440 | 340 |
| Iron (mg) | 9.4 | 5.9 | 6.1 | 6 | 9.4 | 12.5 |
| Zinc (mg) | 3.2 | 4 | 5.5 | 2.5 | 5.1 | 3 |
| Magnesium (mg) | 220 | 150 | 170 | 150 | 100 | 230 |

**Fig. 11.1** Micro-nutrient and vitamin content of ricebean in comparison with other pulses and legumes. Source: Anonymous (2004)

Vitamins such as niacin, riboflavin, thiamine, and ascorbic acid (Joshi et al. 2006) are present in ricebean seed. A laboratory experiment by Buergelt (2009) has also proven ricebean to be a good source of various nutrients. Biochemical constituents of ricebean are carbohydrate 58.2–72%, crude protein 18.3–32.2%, ash 3.5–4.9%, soluble ether extract 0.1–0.5%, and crude fiber 3.6–5.5% (Source: Buergelt et al. 2009) (Fig. 11.1; Table 11.3).

## 11.2.3 Ricebean as a Fodder Crop

Ricebean is a multipurpose underutilized legume. The green forage ricebean is good source of crude protein and crude fiber, which can be fed fresh or processed into hay, and seeds are used as concentrate feed. Ricebean foliage is highly nutritious animal fodder. Ricebean straw after seed harvested, including the stems, leafy portions, empty pods, and seeds can be used as fodder (Singh et al. 2020). Ricebean is valuable as a high-class fodder that is known to increase milk production in livestock.

**Table 11.3** Nutritional composition of ricebean grains

| Component | Content |
|---|---|
| **Crude protein (%)** | 14.00–26.10 |
| **Amino acids (mg/100 g)** | |
| Arginine | 4.32–7.12 |
| Alanine | 3.26–6.60 |
| Aspartic acid | 10.39–13.50 |
| Glutamic acid | 12.36–17.00 |
| Glycine | 2.96–4.26 |
| Lysine | 5.38–8.75 |
| Methionine | 0.90–2.88 |
| Proline | 2.54–8.36 |
| Tryptophan | 1.23–2.00 |
| Tyrosine | 2.12–3.31 |
| Valine | 4.40–5.89 |
| **Minerals (mg/100 g)** | |
| Sodium | 6.00–347.40 |
| Potassium | 610.40–2875.00 |
| Calcium | 111.5–598.23 |
| Magnesium | 73.0–356.12 |
| Zinc | 2.45–10.44 |
| Iron | 3.72–9.25 |
| Manganese | 2.04–5.0 |
| Copper | 0.68–4.97 |
| Phosphorous | 124.0–567.69 |
| **Fatty acid (%)** | |
| Palmitic acid | 5.60–16.88 |
| Stearic acid | 2.10–5.87 |
| Linoleic acid | 7.50–18.98 |
| Oleic acid | 15.62–68.00 |
| Linolenic acid | 39.89–44.38 |

Source: Chandel et al. (1978), Bepary et al. (2017)

### 11.2.4 Importance of Rice Bean in Crop Cultivation

Pulses and legumes are reliable dietary supplements for people of developing countries (Katoch 2013). It is a cheap source of high-quality proteins and adds variety to the palate. Legumes such as moong bean, chickpea, pigeon pea, and lentils are widely cultivated in India and abroad. But the poor availability of soils and various biotic and abiotic stresses have reduced the production and productivity of the crops (Sultana et al. 2014). Farmers and consumers need a cost-effective, stress-tolerant alternative crop for their increasing demands. Crop diversification provides better opportunities for creating a uniform cropping system as well as developing resistance against biotic and abiotic stress. The use of pesticides to mitigate the biotic stresses does not fit well due to higher economic inputs Underutilized legumes play an important role in such scenario providing better alternatives with high degrees of stress tolerance. One such underutilized legume is ricebean (*Vigna umbellata*) which has great potential as an alternative with high nutritional and economic value (Dhillon and Tanwar 2018). Here, the major potential of rice bean as a stress-tolerant crop and physiological aspects under different stress are discussed below.

## 11.2.5 Ricebean on Abiotic Stress

### 11.2.5.1 Response to Drought Stress

Drought can be defined as a period without significant rainfall and it is one of such major abiotic stresses that contributes to a huge reduction in crop yield throughout the world (Vinocur and Atman 2005). Plant shows a broad range of physiological, morphological, and biochemical changes such as reduced photosynthetic accumulation and altered gene expression under the drought stress which ultimately cause reduced growth as well as poor grain yield (Turner 1986; Bray 1993; Baroowa et al. 2016; Maheswari et al. 2016). An adverse effect of drought stress is related to the disturbances of essential physiological properties such as leaf water potential ($\psi$), RWC (relative water content), OP (osmotic potential), SC (stomatal conductance), and TR (transpiration rate) (Anjum et al. 2011; Subramanian and Maheswari 1990a, b; Shanker et al. 2014). Such water imbalance inside the plant system may be responsible for loss in turgor pressure and impairment of cell enlargement.

Osmotic adjustment is the most common form of plant defense under water stress. Accumulation of osmolytes having low molecular mass such as proline, betaine, and polyols is one of the major responses under drought stress (Hanson 1992). Osmolytes play a key role in protecting the macromolecular structures and their functions by adjusting the osmotic balances (Timasheff 1992). Several reports are available for the accumulation of cyclic polyols such as D-pinitol (1d-3-Omethyl-chiro-inositol) and D-ononitol (D-4-O-methyl-myo-inositol) by different plant species including legumes under drought stress (Richter and Popp 1992; Keller and Ludlow 1993; Wanek and Richter 1997). These two substances are methylated derivatives of myo-inositol which is biosynthetically produced from Glucose-6-P by myo-inositol-1-phosphate synthase (m1PS) and myo-inositol monophosphatase (cf. Wanek and Richter 1997; Noiraud et al. 2001). Methylation of myo-inositol is mediated by a methyl transferase enzyme, myo-inositol-6-O-methyl transferase (m60MT) which is encoded by IMT1 gene (Vernon and Bohnert 1992; Rammesmayer et al. 1995; Wanek and Richter 1997).

Wanek and Richter (1997) purified and characterized m60MT from *V. umbellata* and further they reported (1997) a significant increase in ononitol content in the leaves upon imposition of drought stress by withholding water for nine days. However, neither significant accumulation of ononitol nor any activity of m60MT was found in rice bean roots, whereas a remarkable increase in activity was observed in stems. Higher activity of m60MT in stems and greater accumulation of ononitol in leaves suggested possible transport of this substance from stem to leaves (Wanek and Richter 1997). Ricebean, although performing well under humid conditions, was also tolerant to drought (Chatterjee and Mukherjee 1979; Mukherjee et al. 1980; NAS 1979) and high temperatures. Jana et al. (2016) previously reported that Bidhan Ricebean-1 shows tolerance to drought stress.

### 11.2.5.2 Response to Salinity Stress

Salinity is one of the most influential stressors for increasing production in cropping areas throughout the world. It is an environmental factor that also limits crop productivity or damages biomass. Salinity stress in soil occurs when an excess concentration of soluble salts ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$) in the root zone of plants makes constrains to uptake nutrients and water from the soil and causing plant injury (Szabolcs 1989). The injurious effects of salinity on plant growth are specific ion toxicity, nutrition deficiency and attributed to a decrease in osmotic potential of the growing medium (Greenway and Munns 1980). Salinity stress affects plant growth because the high concentration of salts in the soil solution interferes with the balanced absorption of essential nutritional ions by plants (Tester and Davenport 2003). Salinity can affect plants mainly in three ways. Initially salt makes it very difficult for plants to withdraw water from soil due to very low osmotic potential. In effect, the plants suffer from a sort of osmotic stress which restricts plant growth causing yield reduction. Secondly, the $Na^+$ and $Cl^-$ ions taken up by plants from saline water are toxic to plants. Due to the absorption of these ions along with water in high concentration, plants suffer from cytotoxicity, resulting in the reduction of growth, leaf burn, and plant death. Thirdly, the presence of high concentration of Na + and Cl- ion reduces the availability of other ions like $K^+$, $Ca^{2+}$, and $Mg^{2+}$, thus causing other nutritional disorders (Atta et al. 2020, 2021). There are few researchers who have done their work on salinity stress on ricebean.

There are six subgenera under genus *Vigna* (Leguminosae) distributed in Australia, Africa, America, and Asia (Verdcourt 1970; Maréchal et al. 1978). In Asia, most of the *Vigna* species belong to the subgenus Ceratotropis, which is also known as Asian *Vigna* (Tomooka et al. 2002), and ricebean [*Vigna umbellata (Thunb.) Ohwi et Ohashi*] is one of them. Avoidance (excluder type) and tolerance (includer type) are the two mechanisms (Levitt 1980; Munns and Tester 2008) of plant salt tolerance. The "includer type" plants take up $Na^+$ ions with a relatively lower toxicity, while "excluder type" plants exclude toxic ions from internal plant tissue (Johnson et al. 1991). *Vigna* genus only reported "excluder type" salt tolerance mechanisms so far, where *Vigna unguiculata (L.) Walp.* (cowpea) and mung bean prevent $Na^+$ migration from the root to the aerial part or restrict $Na^+$ uptake from the root (Jacoby 1964; Lessani and Marschner 1978; Fernandes de Melo et al. 1994; West and Francois 1982; Bernardo et al. 2006). Yoshida et al. (2016) previously reported, while working on different concentrations of NaCl stress, that *Vigna umbellata* showed slight salinity tolerance at the early stages of seedlings and later on as the duration of stress increases, the tolerance of the salinity became more prominent. The germplasm KRB-272 and KRB-274 was reported by Jana et al. (2017) as resistant to salinity stress when tested with various growth parameters for salinity tolerant index. Atta et al. (2019) while working on Bidhan Ricebean-1 in germinating seeds shows some tolerance to salinity stress when compared with unstressed control.

#### 11.2.5.3 Response to Cold Stress

Cold temperature stress is also a limiting factor, which comes under abiotic stress. Chilling injury and freezing injury are the classified forms of low-temperature injury based upon the severity of cold. Basically, when the temperature remains above freezing point (>0 °C) chilling injury occurs and freezing injury occurs at a temperature below freezing point (0 °C). Both the low-temperature injury caused detrimental effects on plants, which includes cell membrane disarranging, retarded pollen germination and impeded in pollen formation (Steponkus et al. 1993; McKersie and Bowley 1997), photosynthesis disturbances (Bell 1993), disruptions in electron transport chain (Hallgren and Oquest 1990), and also $CO_2$ fixation involved enzymes (Sassenrath et al. 1990). ROS (reactive oxygen species) activities increased as usual due to chilling temperature, as a result chilling injury occurs (Omran 1980; Hodgson and Raison 1991; Prasad et al. 1994).

There are a series of biochemical and physiological changes that involve cold tolerance mechanism, that cause alteration in lipid composition in cell membrane (Graham and Patterson 1982; Murata and Yamaya 1984), increase in ABA (Rikin and Richmond 1976; Ciardi et al. 1997; Morgan and Drew 1997), and also the changes in osmolytes and increase in antioxidants (Fridovich 2006). Low-temperature stress is more frequent in the temperate region making a significant threat to vegetative growth by necrosis of leaf tip, chlorosis, and curling of the whole leaf. Likewise, reproductive stage constitutes the most vulnerable phase where damaging events may take place, such as, the juvenile buds drop, reduced pollen viability, aborted pods and stigma receptivity, reduced pollen tube growth, and finally, deteriorated seed quality and seed yield (Kumar et al. 2007, 2010). There are few research works done till now on cold stress in ricebean. Jana et al. (2016) reported that Bidhan Ricebean-1 shows tolerance to cold stress.

#### 11.2.5.4 Ricebean on Metal Stress

Heavy metals at toxic level on plants trigger a wide range of physiological and metabolic alterations. The most widespread visual evidence of heavy metal toxicity could be a reduction in plant growth (Sharma and Dubey 2007) including leaf chlorosis, necrosis, turgor loss, a decrease in the rate of seed germination, and a crippled photosynthetic apparatus, often correlated with the acceleration of senescence processes and plant death (Dalcorso et al. 2010; Carrier et al. 2003). All these effects are related to ultra-structural, biochemical, and molecular changes in plant tissues and cells brought about by the presence of heavy metals (Gamalero et al. 2009).

#### 11.2.5.5 Response to Aluminum Stress

Al toxicity is a growing concern in modern agriculture, especially for soils having acidic pH (Kochian 1995). Plant productivity can seriously be affected due to the onset of Al stress, even a micromolar concentration is sufficient to limit water and nutrient uptake of many species by rapidly inhibiting root elongation (Yang et al. 2007). However, plants evolved with their own defense mechanism to cope with such abiotic stresses. Major crops like wheat, maize, and soybean are found to display a wide range of variation for Al tolerance that may be utilized to improve other commercially grown susceptible plant species (Ryan et al. 2001). Exclusion of excess Al externally or through internal tolerance mechanism is the most common form of defense (Ma et al. 2001). Tolerant genotypes are reported to release organic anions from their roots to chelate Al ions in the rhizosphere by forming nontoxic complexes (Ryan et al. 2001). Depending on the time of secretion of organic ions from roots as a response to Al stress plants may be broadly categorized into two patterns: Pattern I (secretes organic ions as soon as Al stress emerges) and pattern II (take few hours to respond) (Ma et al. 2000). Wheat, buckwheat, and tobacco follow pattern I to activate organic anion efflux through malate, oxalate, and citrate secretion, respectively, whereas, along with *Cassia tora*, triticale and rye (Ryan et al. 2001), rice bean (*Vigna umbellata*) which is a potent leguminous crop that grows well in acidic soils also can withstand Al toxicity following pattern II citrate secretion from root apex in order to detoxify Al externally (Yang et al. 2006; Fan et al. 2014). The delay in showing such physiological response suggests the presence of some intermediate steps between reception of stimulus and anion efflux (Ma et al. 2000).

Although several Al responsive genes from different species (*Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, *Glycine max*) have been reported so far (Goodwin and Sutter 2009; Yamaji et al. 2009; Tsutsui et al. 2012; Mattiello et al. 2010; You et al. 2011), true association with Al tolerance were recognized for very few genes (Delhaize et al. 2012). Such contradiction was raised probably because of improper characterization of plant symptoms under severe Al toxicity which not only inhibits root growth but also imposes some secondary effects that are difficult to distinguish from the primary targets (Kochian 1995).

Cloning of two Aluminum stress-responsive citrate transporter genes *VuMATE1* and *VuMATE*2 coding for multidrug and toxic compound extrusion (MATE) protein (Yang et al. 2006; Liu et al. 2018) using RACE (Rapid Amplification of cDNA ends) technology opened a new horizon in comprehending molecular mechanism of rice bean Al toxicity tolerance. *VuMATE1* gene transcription at root apex was only observed under Al stress (Liu et al. 2013), although not consequently, rather after 6 h of exposure to Al stress. However, further investigation with a potential role of *VuMATE2* gene in rice bean suggested a biphasic citrate secretion from root apex under Al stress where an earlier expression of *VuMATE2* is reported (Liu et al. 2018). Tissue-specific expression of *VuMATE2* gene was observed under Al stress. 5 μM of external Al concentration was found sufficient to upregulate *VuMATE2* gene throughout the root (both at basal and tip region), whereas no such increase in gene expression was detected in leaf tissues. In addition to the different expression patterns of *VuMATE1* and *VuMATE*2, Liu et al. (2018) also speculated a contrasting signal transduction pathway between these two genes. Where de novo synthesis of a transcriptional activator is supposed to initiate *VuMATE1* transcription (Liu et al. 2013, 2016), $Al^{-}$-induced degradation of repressor supports *VuMATE*2 expression.

Al toxicity also decreases the Mg concentration in root cells. However, Mg plays a crucial role in maintaining ATPase protein ($H^+$-ATPase) activity on plasma membrane (Brooker and Slayman 1983). Micromolar concentrations of Mg have been reported to increase the $Al^-$ stress-responsive citrate efflux in rice bean roots due to the upregulation of Mg-dependent plasma membrane $H^+$-ATPase activity (Yang et al. 2007). Further extension of research in this area may help us to find further explanations of Mg-associated Al stress tolerance in rice bean and other crops as well.

## 11.2.6 Ricebean on Biotic Stress

Biotic stress is stress that occurs as a result of damage done to an organism by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. Plants react to biotic stress by a defense system. Innate response and systemic response are the classified forms of defense mechanism. After infection, generation of ROS occurs and oxidative bursts limit pathogen spread (Atkinson and Urwin 2012). As pathogen attack occurs, plants increase cell lignification and this mechanism blocks invasion of parasites and reduces host susceptibility. The defenses include structural and morphological barriers, proteins, chemical compounds, and enzymes. These converse resistance or tolerance to biotic stresses by protecting products and by giving them strength and rigidity. Crop diversification provides better opportunities for creating a uniform cropping system as well as developing resistance against biotic and abiotic stress. Underutilized legumes play an important role in such scenario providing better alternatives with high degrees of stress tolerance.

### 11.2.6.1 Storage Bruchid Pest Resistance

The bruchid weevils are a serious pest of many leguminous crops. The major species of brucid pests that pose a threat to the cultivation of commercial leguminous crops are *Callosobruchus maculatus*, also known as cowpea weevil; *Callosobruchus chinensis*, azuki bean weevil, and *Callosobruchus analis*, graham bean weevil (Kashiwaba et al. 2003). Most of the ricebean varieties have shown resistance to this weevil attack, especially during the vegetative growth stage (Tomooka et al. 2000). There are two major factors that confer resistance to a crop from insects, namely, antixenosis factor and antibiosis factor (Painter 1951). Antixenosis factor depends upon the seed size, seed coat thickness, seed hardiness, etc., whereas biochemical factors such as enzymes and toxins relate to antibiosis factor (Edwards and Singh 2006). In an experiment conducted by Seram et al. (2016), eight ricebean landraces were taken and inoculated with bruchid pest *C. maculatus*. None of the ricebean landraces were rejected by bruchid species for egg laying and seed coat penetration, although the degree of egg laying varied due to differences in the level of compounds present in the seed coat. Despite such heavy inoculation, very few to no larval emergence were observed. The hatching percentage varied from 39.2% to 49.2% with a mean value of 45.25 ± 3.23%. This proved that antixenosis factor was not responsible for bruchid resistance but antibiosis factors present in the cotyledon. Another experiment showed the same results where the bruchid species penetrated the seed and laid eggs but the larvae died in the cotyledons in the first and second larval stages (Kashiwaba et al. 2003). Srinivasan and Durairaj (2007) conducted experiments to calculate the amount of antibiosis enzymes present in ricebean species. The inhibitory activity was expressed as Trypsin/chymotrypsin/cysteine protease inhibitors (TIU/CIU/CPIU) per gram of sample, where one unit of activity was equivalent to 50% inhibition. The data recorded were as follows: TIU ranged between 1576 and 3120 as compared to 680 in check variety (CO 6). CIU ranged 380–583 as compared to 314 in check variety. CPIU ranged 2061–3069 as compared to 686 in check. The role of protease inhibitors has been well documented by Shukle and Murdock (1983) who reasoned that protease inhibitors were capable of inactivating digestive enzymes of the insects and also reduced the quality of proteins that can be digested. This results in nutritional deficiency in insects which ultimately results in stunted growth and death of the insects. Thus it can be concluded that the resistance attributed to ricebean species is due to protease inhibitors, namely, trypsin, chymotrypsin, and cysteine protease inhibitors.

#### 11.2.6.2 Yellow Mosaic Virus Resistance

The Yellow Mosaic Virus is a serious disease of most leguminous plants, especially mungbean (Nene 1973). The disease is caused by a begomovirus with bipartite genome. It is a white-fly transmitted single-stranded DNA virus (Khattak et al. 2000). The symptoms of the disease emerge as yellow specs on the leaves near the veins, which later turn chlorotic as the disease progresses (Qazi et al. 2007). YMV infection at the reproductive stage produces either empty pods or yellow colored pods with unviable or infected seeds (Sehrawat and Yadav 2014). Various studies have shown that ricebean is significantly resistant to YMV attack (Kashiwaba et al. 2003; Sudha et al. 2015). Many approaches to incorporate resistance gene from *Vigna umbellata* to other susceptible *Vigna* species have been quite successful. Studies have shown significant cross-compatibility of ricebean with *Vigna mungo* and *V. sublobata* (Sehrawat and Yadav 2014). Genetic similarity, same chromosome number ($n = 11$), and other morphological similarities between ricebean and mungbean (*V. radiata*) allow breeders to make successful interspecific crosses (Bharathi et al. 2006; Pandiyan et al. 2010; Chaisan et al. 2013; Bhanu et al. 2017; Mathivathana et al. 2019). Modern approaches such as Marker Assisted Breeding (MAB) give pace to the conventional breeding taking advantage of molecular markers (Ashraf and Foolad 2013). Micro-satellite markers or commonly known as SSRs (Simple Sequence Repeats) are widely used in the hybridity confirmation, phylogeny, gene maps, and associated marker-traits establishment in a segregating population. This further helps in selecting specific genes for resistance to incorporate in desired species (Michelmore et al. 1991). In a research conducted by Mathivathana et al. (2019), a population of 108 inter-specific recombinant inbred line (RIL) population from a cross between VRM (Gg) 1(Mungbean) and TNAU RED (Ricebean), designated VRMTNAU, was developed and used to construct the genetic linkage map and detect the QTLs.

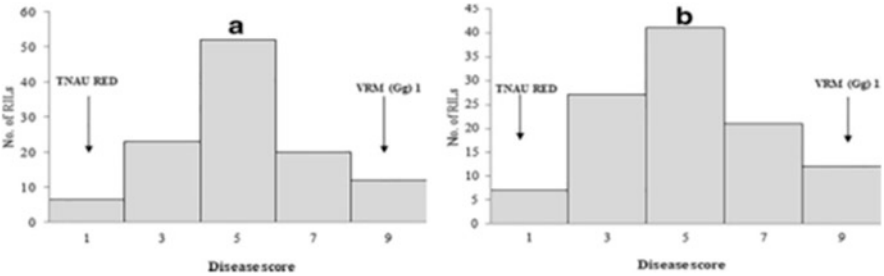


**Fig. 11.2** Frequency distribution of MYMV resistance in $F_9$ RIL population derived from the cross between VRM (Gg) 1 and TNAU RED. (**a**) 2015 and (**b**) 2016 (Mathivathana et al. 2019)

Normal distribution of pattern (Fig. 11.2) obtained by phenotypic disease scoring (1–9 scale) of the RIL population confirmed the quantitative control of the resistance gene. The RIL population was also found to have high heritability of MYMV incidence with significant consistency for two consecutive years (0.75 and 0.78, respectively). A total of four QTLs were detected, namely, qMYMV4_1, qMYMV5_1, qMYMV6_1, and qMYMV10_1 distributed on chromosomes 4, 5, 6, and 10. The QTL on chromosome 4 (qMYMV4_1) was identified as the major QTL, present in all segregating populations consistently, showing resistance. Using mungbean genome as the reference, 83 annotated genes coding for known, unknown, or hypothetical proteins were found to be associated with the qMYMV4_1 region. It was observed that none of the resistant lines encoded the nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins. The R gene proteins that were encoded were serine/threonine protein kinase super family; MYB transcription factor; WRKY family transcription factor; and zinc finger RING/FYVE/PHD-type protein. A few small protein-encoding sites were also observed for small GTP-binding protein, receptor-like kinase (RLKs) protein, and jasmonic acid carboxyl methyltransferase (JMT) proteins. In conclusion, it was observed that about four QTLs were responsible for disease resistance against the virus. Of these qMYMV4_1 was a stable and major QTL for resistance to YMV. Therefore, further exploration may open the prospect for gene cloning followed by introgression to other cross-compatible species as well to facilitate crop improvement.

## 11.3 Conclusion

Clearly, ricebean is a potentially valuable multipurpose (grain, fodder, and green manure) crop for farmers in the marginal hill areas of Nepal and northern India, as well as in third countries with similar environments. Considering ricebean as a climate as well as pest resilient crop it can be concluded that this crop has tremendous potential in agronomic cultivation as well as in scientific research. Biotic as well as abiotic stress resistance can be incorporated into the background of other commercially important legumes to improve their defense against different stresses. Future research may be focused on further elaboration of molecular mechanism underlying drought tolerance of ricebean. Germplasm collection and screening for other stress resistance will pave the way for further research of this crop. Additionally, rice bean cultivation as an economically important crop can also be promoted by growing interest among the farmers for this pulse crop.

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