Indian J. Agric. Res., 53(2) 2019: 223-227
Print ISSN:0367-8245 / Online ISSN:0976-058X

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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Tolerance mechanisms of Indonesian plant varieties of yardlong beans (*Vigna unguiculata* sub sp. *sesquipedalis*) against drought stress

M.W. Lestari*, N. Arfarita, A. Sharma[1] and B. Purkait[2]

Faculty of Agriculture,
University of Islam Malang, East Java, Indonesia.
Received: 26-05-2018 Accepted: 27-02-2019 DOI: 10.18805/IJARe.A-369

ABSTRACT

Plants that experience drought stress protects themselves through several mechanisms namely avoidance, tolerance, recovery, and escape. Eight varieties of yardlong bean plants were subjected to a drought stress at the rates of 50% of the field capacity (FC) and 100% of the field capacity (FC). The results showed that yardlong bean plants possessed the mechanisms of escape, demonstrated by the increase in Peroxidase (POX) enzyme activity. Avoidance was demonstrated by the reduced evapotranspiration surface of the decreased leaf surface area, and the increased number of closed stomata. Tolerance was demonstrated by the increased proline accumulation.

Key words: Avoidance, Drought stress, Escape, Indonesian yardlong beans, Tolerance.

INTRODUCTION

Yardlong beans are an important horticulture crop in Indonesian and an agricultural commodity where its demand exceeded significantly day by day (Kuswanto, 2002). Whereas low productivity supposed due to the continuing cultivation of varieties that have sub-optimal yields and poor drought tolerance, along with the limited area under cultivation. On the other hand, yardlong beans also have high economic value with fairly stable prices.

The production of yardlong beans in Indonesia in the years 2014-2016 was 450,712 tons, 395,524 tons, and 388,059 tons respectively (Statistics Indonesia, 2017). There is the unavailability of leading varieties capable of high yield, with resistance to drought stress and pests. Drought stress significantly affected all assessed traits under drought condition. Catalase enzyme, chlorophyll content and Relative Water Content were reduced, but, peroxidase enzyme and Proline content, as well as electrolyte leakage increased and the highest content was observed under severe drought stress 40% FC (Noori, *et al*., 2018). Drought stress is an environmental threat that most likely limits growth and production, and are serious threats to crop productivity losses than any other abiotic stress factor (Mahantesh, 2018).

Plants can cope with drought either through escape or avoidance. Escaping drought entails the completion of the life cycle in advance of the effects of drought (Heschel and Riginos, 2005; Wu *et al*., 2010). By contrast, plants avoid drought by maintaining high fitness despite drought conditions. This strategy sometimes referred to as drought tolerance (Heschel and Riginos,2005). Although plants could potentially escape and avoid drought, theory and previous findings suggest that there are likely to be trade-offs between these strategies (McKay *et al*., 2003; Heschel and Riginos, 2005). The reason for this is that a trait that allows for greater drought avoidance, such as stomatal closure, the decline in leaf area or another agronomic physiological response. Drought can also potentially cause either evolutionary changes in avoidance or escape such as peroxidase antioxidant enzyme or faster early flowering. Peroxidase (POX) to destroy ROS (Reactive Oxygen Species) (Harinasut *et al*., 2003). Compared to more sensitive plants, the accumulation of proline in drought-tolerant plants is higher. The POX enzyme plays a role in controlling the level of oxidative stress and is related to tolerance against abiotic threats, including drought stress because this enzyme can reduce H_2O_2 into water (Mohammadkhani and Heidari, 2007).

With plasticity, the expression of the phenotype is shaped by environmental conditions. A plasticity response to drought would mean that the plants alter their phenotype by increasing avoidance or escape traits in drought relative to non drought conditions (Mal and Lovett-Doust, 2005; Caruso, 2006). By contrast, the drought could also act as an agent of selection and cause genetically based evolutionary changes in avoidance or escape (Ludwig *et al*., 2004).

This research is aimed to identify and understand the tolerance mechanisms by which Indonesian yardlong bean plants face drought stress.

*Corresponding author's e-mail: mwlestari@unisma.ac.id
[1]Indian Institute of Technology Kharagpur, West Bengal, India.
[2]Department of Biochemistry, Midnapore Medical College, West bengal, India.

Table 1: Phenotypic specification of yardlong beans.

Nama of variety	Year of release	Color of pod
Brawijaya Ungu-1 (BU-1)	2015	Purple
Brawijaya Ungu-2 (BU-2)	2015	Purple
Brawijaya Ungu-3 (BU-3)	2015	Purple
Brawijaya Ungu-4 (BU-4)	2015	Purple
Brawijaya Ungu-5 (BU-5)	2015	Purple
Brawijaya Ungu-6 (BU-6)	2015	Purple
Brawijaya-4 (Br-4)	2010	Light green
Bagong-2 (Bg-2)	2010	Light green

MATERIALS AND METHODS

Experimental design: The data analysis using the Factorial Randomized Complete Block Design. The plants were grown in the greenhouse of the Faculty of Agriculture, University of Islam Malang, East Java Indonesia during the period of March to June of 2016, 550 m above sea level, temperature 20° – 29° C, 112°06' – 112°07' east longitude and 7°06' – 8°02' north latitude. The first objective consisted of 8 varieties of yardlong beans, released by Indonesia Government and commercial available in local market. The specification of phenotypics this yardlong beans showed in Table 1.

The second objective was varietal screening against drought stress at the rates of 100% field capacity (FC) and 50% field capacity (FC). All yardlong bean cultivates in pot under controlled condition in greenhouse were watered until they reached the age of 30 days after sowing. For drought stress treatment, the plants were stop water after these achieved the moisture content of 100% FC. Maintained the 100% FC as control watering was given when the moisture content was less than 100% FC for the control while less than 50% FC for the drought stress treatment.

Parameters measured to analyze drought stress of the plants: The evaluation of the growth of the yardlong bean plants was performed by measuring: were leaf area, measured through number of closed stomata, leaf proline content (Bates *et al.*,1973) and POX activity, as observed on the third leaf from the tip of the plant (Panda *et al.*, 2003).

Data analysis: All data were analyzed statistically using ANOVA on *SPSS Release 15*. If the treatment had significant effects, it was then followed with a Duncan t-test performed at a confidence level of 95%.

RESULTS AND DISCUSSION

The effects of drought stress on escape mechanism: Drought stress caused a significant reduction in quantified by the start of flowering (Fig 1). Drought stress caused all varieties of yardlong beans to flower quickly by 2 to 5 days. The variety that flowered the fastest was Brawijaya Ungu-4 (10% faster), whereas the variety that flowered the slowest was Bagong-2 (2% faster). This showed that the yardlong bean plants used the escape mechanism to adapt to conditions of drought stress. The results of this research correlate with that of Mitra (2001); that is, many plants accelerate the flowering age and harvest age (accelerating its life cycle) to escape from drought stress. Yardlong bean plants demonstrated a drought escape mechanism, which is the ability of a plant to complete its life cycle before experiencing a severe deficit of water. This mechanism is indicated by fast flowering, which refers to a plant's ability to maintain the potentials of its tissue by increasing water absorption or suppressing water loss.

The analyses of results on yardlong bean leaves showed that the drought stress treatment caused an increase in POX enzyme activity (Fig 2). However, the increase in POX enzyme activity was different for each of the varieties. The highest increase in POX enzyme activity occurred to variety Brawijaya Ungu-3 (340 %), whereas the lowest occurred to Brawijaya Ungu-4 (28 %). Harinasut *et al.* (2002) reported in a research study that the enzymes responsible for the detoxification of hydrogen peroxide are peroxidase and catalase. However, peroxidase has a more significant role than catalase in this detoxification process because hydrogen peroxide is involved in peroxidase-mediated oxidative polymerization, which causes a strengthening of cell walls. The activation of peroxidase plays a role in protecting cells. The increase of peroxidase activity under drought stress conditions is associated with protection against

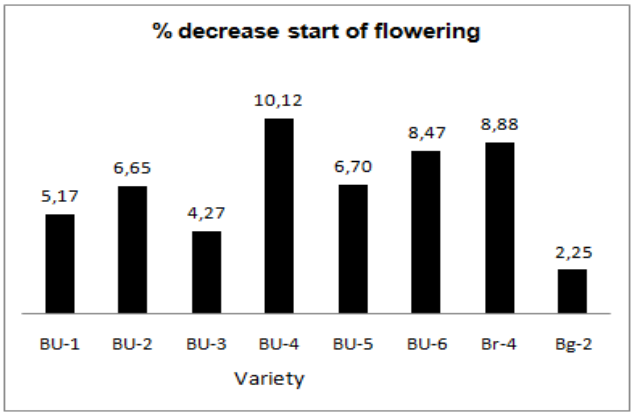


Fig 1: The decrease start of floweringof some varieties of purple yardlong beans due to drought stress.

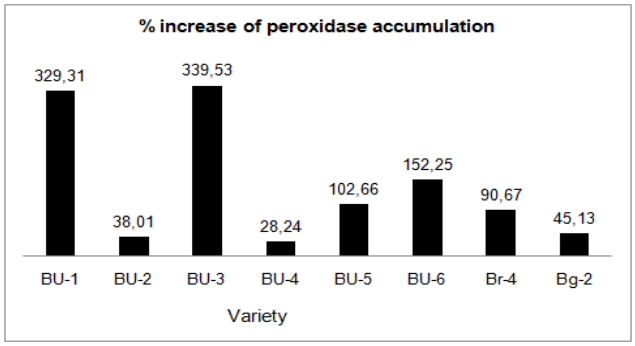


Fig 2: The increase level of peroxidase accumulation of some varieties of purple yardlong beans due to drought stress.

oxidative damage, lignification, and cross-linking of cell walls; thus, it serves to prevent more severe cell damage (Moussa and Abdel-Aziz, 2008).

The effects of drought stress on tolerance mechanism: Fig 3 showed that drought stress caused a significant reduction in leaf relative water Content (RWC). Leaf water content can be used as measures of the physiological impact of water stress on plant performance. Leaf relative water content decreases due to drought stress for all varieties. The largest decrease occurred to variety Brawijaya-4 (55%), whereas the varieties that experienced the least decrease were Brawijaya Ungu-3 (13%) and Brawijaya Ungu-6 (15%).

All the tested varieties of yardlong beans showed an accumulation of proline due to drought stress and each Brawijaya Ungu variety possessed different accumulation levels of proline (Fig 4). This showed that each of the varieties had differing levels of tolerance. Brawijaya Ungu-1 experienced the highest increase in proline accumulation (514%) and was significantly different from the other varieties. As for Brawijaya Ungu-2, it experienced the lowest increase of proline accumulation (56%), although it was not significantly different from that of Brawijaya-4 (63%).

Prior researches had proven that proline accumulation had several advantages for the cells. Proline can function as a source of energy, nitrogen, and carbon, as well as an osmolyte in response to drought stress; additionally, proline could also reduce free radicals in the cells and thus can prevent damage due to oxidative stress. Proline is also involved in the protection of enzymes from becoming denatured and can interact with the membrane system, regulate the acidity (pH) of the cytosols, and regulate the balance between NADH/NAD^+ (Konstantinova *et al.*, 2002).

The increase of proline content in the leaf was also found in plants that are tolerant to salinity (Delauney and Verma, 1993). Lobato *et al.* (2008) explain that a mechanism such as this is recognized as plant tolerance toward drought stress through an accumulation of dissolved substances, which in this case is the accumulation of leaf proline content. The increase of proline content in plants acts as an osmotic regulator that involves the accumulation of dissolved substances.

The effects of drought stress on avoidance mechanism: All yardlong bean plants experienced different degrees of increase in the number of closed stomata due to drought stress (Fig 5). The apparent effects were different among the tested varieties. The number of closed stomata increased in the varieties given drought stress treatment. The highest increase in the number of closed stomata occurred to variety Brawijaya Ungu-3 by 161%, which was significantly different from other varieties. As for the lowest increase, it occurred to varieties Brawijaya Ungu-2, Brawijaya Ungu-4, and Brawijaya Ungu-6 by 35%, 36%, and 36%, respectively.

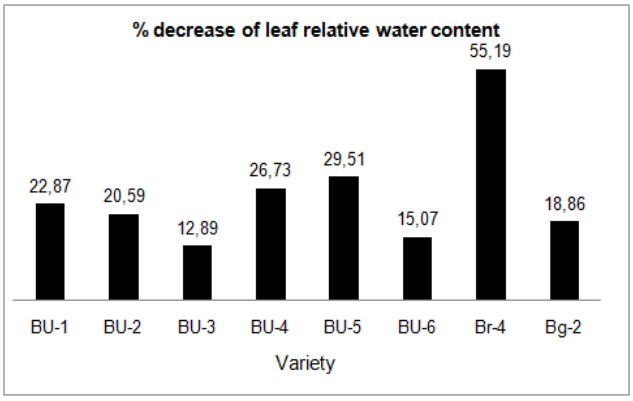


Fig 3: The decrease level of leaf relative water content of some varieties of purple yardlong beans due to drought stress.

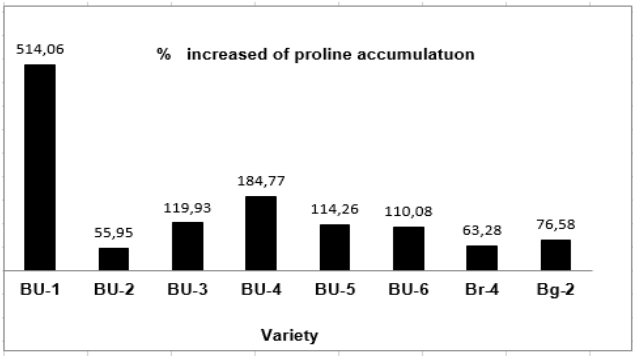


Fig 4: The increase level of proline accumulation of some varieties of purple yardlong beans due to drought stress.

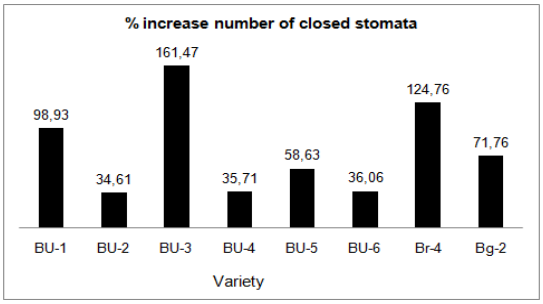


Fig 5: The increase level number of closed stomata of some varieties of yardlong beans due to drought stress.

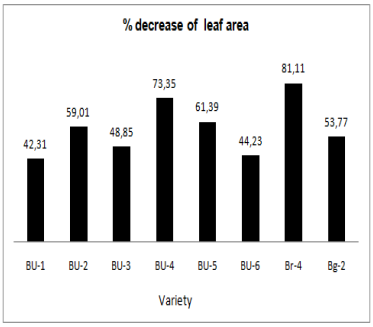


Fig 6: The decrease level of leaf area of some varieties of purple yardlong beans due to drought stress.

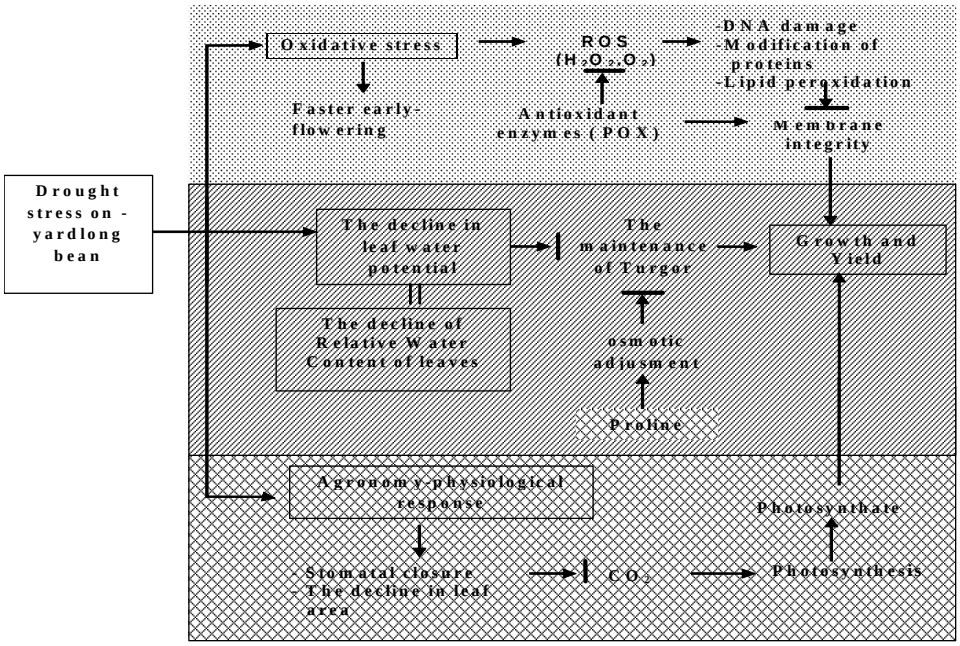


Fig 7: Yardlong bean mechanism in facing drought stress and its impact on growth and yield.

Note: Indicates tolerance mechanism of *escape*
Indicates tolerance mechanism of *tolerance*
Indicates tolerance mechanism of *avoidance*

Brodribb and Holbrook (2003) stated that under drought stress conditions, stomata will close because of the accumulation of abscisic acid (ABA) and because of the interaction with elevated temperatures. Further, Kalefetoğlu and Ekmekci (2005) stated that, during drought stress conditions, the rate of photosynthesis decreased due to the closure of stomata as well as the reduction of electron transport and phosphorylation capacity in the leaf chloroplasts.

According to Borges, Barbosa, and Nahas (2013), and Purwanto (2003), plants under drought stress conditions would close their stomata to conserve water and to avoid dehydration, which in turn inhibited transpiration, reduced photosynthesis, and eventually decreased assimilation and pod productivity (Basal and Unay, 2009). The important physiological indicator in drought stress tolerance was the reduction of leaf transpiration through the stomata (stomatal transpiration) and cuticles (cuticular transpiration).

Based on the results from observing physiological characteristics, yardlong bean plants possess the mechanism of avoidance, which refers to the ability of plants to maintain tissue potential by increasing water absorption or suppressing water loss by increasing the number of closed stomata.

Leaf area of the plants decreased during the onset of the drought stress (Fig 6). The reduction of leaf area represented one of the perceptible plant reactions from the lack of moisture at the start of vegetative growth. The reduction in leaf area constituted an escape mechanism in an effort to suppress the loss of moisture, by reducing transpiration. The small leaf size is very important in maintaining the turgor potential (Widyasari*et al*., 2009). The loss of turgidity will arrest cell development (replication and enlargement) and will, in turn, inhibit the increase of leaf area (Kisman, 2010). In this research, the smallest reduction of leaf area occurred to Brawijaya Ungu-1 (42%), whereas the greatest reduction of leaf area occurred to Brawijaya-4 (81%). This showed that based on the agronomic characteristic of leaf area, Brawijaya Ungu-1 was more tolerant to drought stress, whereas Brawijaya-4 was more sensitive toward drought stress. Yardlong bean plants demonstrated a drought avoidance mechanism, with this mechanism, yardlong bean plants showed a reduction in evapotranspiration surface through the decrease of leaf area. The tolerance mechanism of yardlong bean plants in facing drought stress can be seen in Fig 7.

CONCLUSION

In the face of drought stress, yardlong bean plants of the varieties Brawijaya Ungu possessed mechanisms that include: a) escaping by ending its growth as quickly as possible, which was indicated by the fast formation of flowers and the increase of POX enzyme activity to inhibit ROS; b) avoiding the effects of water shortage in such environments, which was indicated by the decrease of evapotranspiration surface through the decrease of leaf area and increase in number of closed stomata; and c) tolerating low tissue water potential, which was indicated by the increase of proline accumulation.

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