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Screening of efficient drought tolerant accessions of *Terminalia arjuna* plant for sustainable Tasar sericulture production based on biochemical tools

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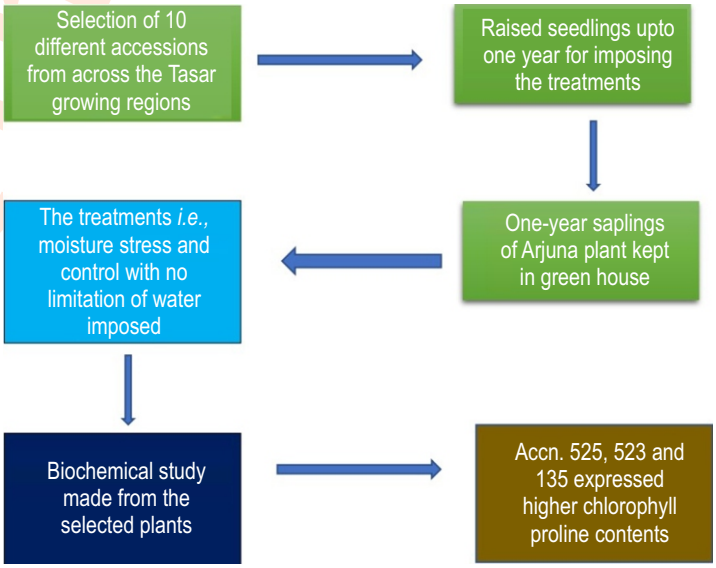
Abstract

Aim: Tasar silkworm, *Antheraea mylitta*, feeds on the foliage of many plant species belonging to different families, but it prefers the foliage of *Terminalia arjuna*, *T. tomentosa* and *Shorea robusta*. The aim of the present study was to screen the drought-tolerant accessions of Arjuna for the successful and sustainable rearing of the Tasar silkworm.

Methodology: An investigation was undertaken at the Central Tasar Research and Training Institute, Ranchi during 2016–17 and 2017–18 to study the "screening of fast-growing drought tolerant accessions of Arjuna for raising block plantations" by using biochemical tools. Ten accessions (40 saplings from each accession) were selected and allowed to grow for one year. Then, drought treatments were imposed. The experiment was laid out in a factorial completely randomised design (FCRD) with two factors. Two factors were accessions and moisture levels, i.e., control and stress.



Results: The experimental result revealed that the *T. arjuna* accessions differed significantly for total chlorophyll contents under drought stress. Accn. 523, 525 and 135 significantly recorded higher chlorophyll content under drought-stress conditions. Water stress increased the proline concentrations in all accessions of *T. arjuna*, reaching 3–16 fold over the non-stressed control. Proline level was significantly increased in Accn. 525 followed by 523 and 135 at 7 and 14 days after treatment was imposed.

Interpretation: Accessions of *T. arjuna* plant 525, 523, 123 and 135 can be used as the most drought tolerant accessions under drought conditions with limited leaf yield and quality loss.

Key words: Biochemical tools, Chlorophyll, Drought, Proline, Tasar silk, *Terminalia arjuna*

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Introduction

India has the distinction of being the only country in the world to produce all five commercially exploited silk varieties, *viz.,* Mulberry silk, Tropical and Temperate Oak Tasar, Eri Silk, and Muga. Tasar (tropical tasar) silk, largely found in the tribal habitats of the Gondwana region, is a peculiarity of India and not found anywhere else in the world. In India, Tasar silk is largely produced in Jharkhand, Chhattisgarh, and Orissa, besides Maharashtra, West Bengal and Andhra Pradesh. Tasar culture has the immense potential of providing employment opportunities to a large tribal population with little investment. Tasar silkworm, *A. mylitta*, feeds on the foliage of many plant species belonging to different families. Still, it prefers the foliage of *Terminalia arjuna*, *T. tomentosa* and *Shorea robusta* which are designated as primary food plants. *Terminalia arjuna* (Arjuna) is a primary food plant for the Tasar silkworm, growing in the wild areas of India, mostly in the Indian states of Jharkhand, Chhattisgarh, Odisha, West Bengal, etc.

This plant is largely grown in rainfed situations. Now the government of some Indian states is promoting this plant as growing under block plantations to carry out economical tasar sericulture. Water availability in rainfed areas is not appreciable for this plant, whether in the form of moisture stored in the soil or during monsoon period. In these situations, Arjuna plantations encounter grave leaf yield losses due to terminal water stress (Toker *et al.,* 2007; Yadav *et al.,* 2006). Drought stress is one of the major environmental constraints for the Arjuna plant, which causes up to 50% leaf yield losses and affects several leaf quality facets. Drought is an intricate abiotic stress, that affects different morpho-physiological and biochemical actions in plants. Proline, glycine betaine (GB), and soluble sugar builds in the cytoplasm when there is a scarcity of water, aiding in osmotic adjustment and enabling the plant to withstand the negative effects of oxidative stress in plants (Elansary *et al.,* 2020). These features can be used to identify the accessions of Arjuna plants for drought tolerance. Several processes or mechanisms have been advocated to adapt to water stress, including a decrease in water loss by increasing stomatal resistance, water uptake by forming large and deep root systems, and accumulation of some biochemicals.

The biochemical osmolytes accumulated include amino acids such as proline (mannitol, sorbitol, and trehalose), sugars, glycine-betaine, and glutamate. These compounds play a vital role in preventing the disintegration of membrane and inactivate of enzymes in a low-water-activity environment. Accumulation of proline due to water stress results from stimulated synthesis inhibited degradation, or impaired incorporation of proline into proteins (Heuer, 1999). Besides, proline plays a more intricate role in determining drought tolerance than it does in acting as an osmolyte (Szabados and Savoure, 2009). Proline is a key component of antioxidant defence system that defend cells from the damage caused by drought stress (Sahebi *et al.,* 2021). Under stressful circumstances, according to Manuchehri and Salehi (2014), the number of soluble sugars, proline concentration, and enzyme activity that scavenges free radicals all increases dramatically to prevent the buildup of reactive oxygen species. The cultivars that have a better ability to produce these biochemicals in response to water stress have a relatively superior ability to produce larger yield (Basu *et al.,* 2016). Water stress is acknowledged to inhibit photosynthetic activity in plant tissues due to an imbalance between light capture and its utilisation (Zhang *et al.,* 2020), resulting in a severe reduction in the chlorophyll contents of the plant. Screening of appropriate characteristics of plants for identifying large number of accessions in a limited time at critical growth stages of the plant to select drought-tolerant accessions remains a key encounter for the plant breeder. Tasar sericulture production must be sustainable in a drought-affected environment in order to ensure the livelihood of farmers, especially the tribal people who practise tasar sericulture. The major objective of this study was to investigate the potential for introducing drought-tolerant Arjuna accessions with higher biomass yield performance in areas where tasar sericulture is primarily practised by the farmers.

Materials and Methods

The study was conducted at Central Tasar Research and Training Institute (CTR&TI), Ranchi, India. The climate of the site is characterised by maximum and minimum temperature ranges of 29.3 °C to 41.7 °C and 4.5 °C to 19.8°C, respectively. The mean annual rainfall during the study period was 1320 mm. The ten most superior accessions of *T. arjuna* were selected in the study for the collection of cutting propagules during the rainy season (Table 1). Arjun plant saplings were raised through the propagation of juvenile and leaf node cuttings from 1-2 month-old branches of the mother plants.

The saplings were allowed to grow in earthen pots for one year. After one year of growth, the saplings were used for further experimental study, *i.e.,* drought tolerance. Drought-tolerant treatments included control and stress treatments. In both treatments, water was fully supplied during the sapling and growth periods to keep the plants growing under well-watered conditions. Thereafter, the water supply was withdrawn and the saplings were allowed to wilt, subjected to water stress in the

Table 1: Selected superior accessions of plant *Terminalia arjuna* and location of their collection

Accession No.	Native of accession
718	Orcha, Madhya Pradesh
135	Sundargarh, Odisha
102	Gadchiroli, Maharashtra
115	Haldi, Uttarakhand
123	Ranchi, Jharkhand
614	KametyaPauri, Garhwal, Uttarakhand
525	Medak, Andhra Pradesh
507	RankaGarwah, Jharkhand
523	Purniyadeeh, Palamu, Jharkhand
424	Haridwar, Uttarakhand

stress treatment. In the case of control treatment, plants of all accessions were maintained with ideal soil moisture throughout the study period. Experiments were terminated after 14 days of water stress. All experiments were conducted under controlled environmental conditions in a greenhouse. Free proline content in a control and stressed Arjuna plant was determined according to the method of Bates *et al.* (1973). Chlorophyll 'a', 'b' and total chlorophyll contents were estimated following the method of Arnon (1949). All the observed data were analysed statistically by analysis of variance. The significance of treatment differences was judged by 'F' test as outlined by Cochran and Cox (1963). Descriptive statistics were applied to analyse the good preferences of two populations in similar environmental situations.

Results and Discussion

Chlorophyll is considered the major chloroplast component for photosynthesis. The chlorophyll content of the leaf is an indicator of the photosynthetic capacity of plant tissues. The results showed that *T. arjuna* accessions differed significantly for chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents under drought stress. Accn 525 showed higher Chlorophyll 'a' and Chlorophyll 'b' contents throughout the experimental periods, irrespective of moisture environmental conditions (Fig. 1,2), followed by Accn 523, which recorded the next higher content. However, Accn 523 showed a higher chlorophyll 'a' content during the initial period and chlorophyll 'b' during 14 DAT under stress environment. Regarding total chlorophyll content, the highest total chlorophyll content was observed significantly in Accn 135, followed by Accn 525 and 115 under control conditions, however, higher chlorophyll content was observed with Accn 525 at 7 DAT and Accn 523 and 525 at 14 DAT. The lower chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were observed in Accn 102 at the initial period and Accn 614 at days 7 and 14 DAT. During the initial period, Accn 523, 525 and 135 significantly recorded the same chlorophyll content under drought stress conditions. However, Accn 525 exhibited the maximum total chlorophyll content, followed by accession 523 and 135 at 7 and 14 DAT, whereas other accessions showed a certain degree of reduction in the chlorophyll pigment under drought stress (Table 2). The highest chlorophyll content under control was recorded in Accn135, 525 and 523 in the initial, 7 and 14 DAT, respectively. Accession 614 and 718 had lower chlorophyll content during the entire study period. Fourteen days after treatment a significant increase in chlorophyll content was noted in Accn 525, 523 and 135 by 73.40, 68.78 and 51.90%, respectively, compared to Accn 614. There are several reports of decreased chlorophyll levels under stress in different species. Chlorophyll and other photosynthetic pigments are negatively affected in the majority of plant species by water deficits caused by drought (Muhammad *et al.,* 2021).

The photosynthetic rate, leaf area, and eventual yield decreased as a result of stress (Sharma *et al.,* 2020). A decrease in total chlorophyll with Accn. 614 under drought stress implies a lower capacity for light harvesting. Since the production of reactive oxygen species (ROS) is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Herbinger *et al.,* 2002). This decrease in total chlorophyll in Accn 614 and 718 were also consistent with the reduced level of lipid peroxidation during stress conditions. The findings are in line with those of Bai and Purcell (2021) who reported that water scarcity induced a considerable decrease in chlorophyll a and b levels in six cultivars of *Triticum aestivum*. Several species have had decreased or stable chlorophyll levels during drought stress, depending on the length and intensity of the drought (Dubois and Inze, 2020). Also, with decreasing chlorophyll content due to the changing green colour of leaf into yellow, the reflectance of the incident radiation

Table 2: Variability of total chlorophyll content (µg ml^{-1}) of different *T. arjuna* accessions as a function of non-stress and drought stress-controlled environment conditions.

Accessions	Initial		7 DAT		14 DAT	
	Control	Stress	Control	Stress	Control	Stress
718	20.16 ±0.14	19.97 ±0.05	21.69 ±0.09	20.72 ±0.04	20.90 ±0.06	17.13 ±0.07
135	25.69 ±0.22	28.21 ±0.04	26.11 ±0.18	25.90 ±0.09	25.58 ±0.17	22.04 ±0.04
102	19.38 ±0.29	24.31 ±0.61	22.45 ±0.07	24.78 ±0.37	21.39 ±0.19	19.02 ±0.08
115	24.85 ±0.47	25.51 ±0.09	24.69 ±0.16	24.80 ±0.22	22.81 ±0.09	20.21 ±0.11
123	23.80 ±0.18	25.36 ±0.55	28.08 ±0.07	24.49 ±0.35	24.22 ±0.12	20.19 ±0.09
614	21.60 ±0.19	18.05 ±0.10	17.95 ±0.06	18.68 ±0.22	19.22 ±0.06	14.51 ±0.07
525	25.20 ±0.31	28.98 ±0.07	33.26 ±0.17	30.13 ±0.09	26.68 ±0.10	25.16 ±0.17
507	21.59 ±0.14	24.45 ±0.21	22.81 ±0.10	24.11 ±0.17	22.30 ±0.07	19.35 ±0.09
523	23.86 ±0.38	29.00 ±0.20	27.94 ±0.17	27.90 ±0.11	26.88 ±0.08	24.49 ±0.18
424	22.54 ±0.23	21.24 ±0.04	21.60 ±0.06	22.91 ±0.10	21.21 ±0.02	18.59 ±0.13
LSD over A	0.56		0.34		0.22	
LSD over M	0.25		0.15		0.10	
A×M	0.79		0.49		0.31	

A: Accession; M: Moisture; DAT: Days after Treatment. Values are mean ±S.D.

Table 3: Variability of total proline content (µg g^{-1}) of different *T. arjuna* accessions as a function of non-stress and drought stress-controlled environment conditions.

Accessions	Initial		7 DAT		14 DAT	
	Control	Stress	Control	Stress	Control	Stress
718	1.91 ±0.02	1.99 ±0.04	4.30 ±0.06	8.79 ±0.08	5.11 ±0.06	14.77 ±0.10
135	5.83 ±0.04	5.75 ±0.08	7.27 ±0.10	53.04 ±0.36	9.90 ±0.07	129.2 ±0.98
102	3.54 ±0.04	3.60 ±0.04	5.75 ±0.04	16.54 ±0.05	8.24 ±0.05	22.03 ±0.09
115	4.71 ±0.04	4.21 ±0.09	6.22 ±0.10	24.37 ±0.34	9.03 ±0.03	80.55 ±1.32
123	5.34 ±0.07	5.13 ±0.03	7.40 ±0.11	36.68 ±0.65	8.88 ±0.02	101.0 ±1.76
614	1.39 ±0.01	1.60 ±0.02	3.48 ±0.04	9.20 ±0.03	5.07 ±0.06	14.17 ±0.08
525	5.91 ±0.08	5.97 ±0.07	8.62 ±0.21	70.12 ±0.48	10.90 ±0.13	160.2 ±1.67
507	4.07 ±0.02	3.70 ±0.03	5.73 ±0.08	20.84 ±0.26	8.49 ±0.11	49.60 ±0.23
523	5.88 ±0.03	6.05 ±0.02	7.73 ±0.12	53.55 ±0.94	10.18 ±0.07	145.3 ±1.74
424	3.05 ±0.06	2.69 ±0.04	4.96 ±0.06	14.55 ±0.06	6.77 ±0.10	46.74 ±0.08
LSD over A	0.10		0.63		1.55	
LSD over M	0.04		0.28		0.69	
A×M	0.14		0.90		2.19	

A: Accession; M: Moisture; DAT: Days After Treatment. Values are mean ±S.D.

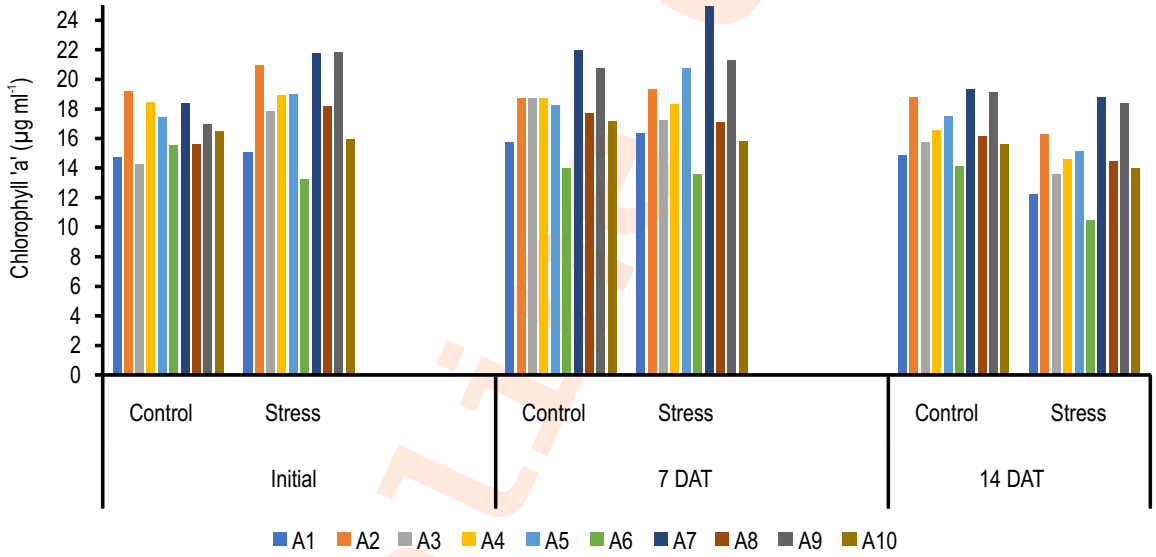

Fig. 1: Variability of chlorophyll 'a' content (µg ml^{-1}) of different *T. arjuna* accessions as a function of non-stress and drought stress-controlled environment conditions. A1:718, A2:135, A3102, A4:115, A5-123, A6:614, A7-525, A8-507, A9-523, A10-424.

increases. Strong drought stress prevents plants from photosynthesis by affecting the quantity of chlorophyll present, disrupting chlorophyll components, and impairing the photosynthetic mechanism (Mafakheri *et al.,* 2010). Schlemmer *et al.* (2005) also pointed out that stressed-susceptible plants fail to absorb enough light radiation due to degradation of chloroplasts, resulting in less chlorophyll content.

Proline, an amino acid, is a compatible solute involved in cell osmotic adjustment and protection of cell components during dehydration. Water-stressed plants of all plant species accumulate a greater amount of proline content than non-stressed plants. The greater proline content found in water-stressed plants suggest an effective method for osmotic regulation, stabilisation of sub-cellular structures, and cellular adaptation to water stress, which is consistent with the earlier studies (Rajendrakumar *et al*., 1994; Hare and Cress, 1997; Gunes *et al.,* 2008). Hence, it is claimed that proline accumulation help the plants to adapt under drought stress. Different genotypes exhibit distinct patterns of proline accumulation under drought stress. A strong relation between proline accumulation volume and drought tolerance has been proposed as a measure of

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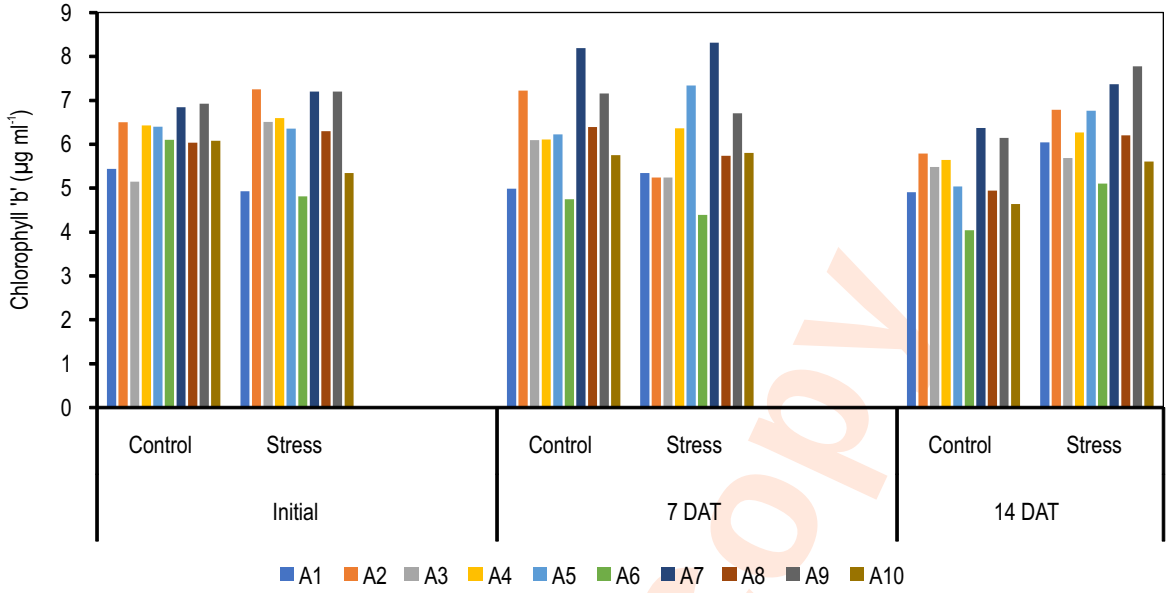

Fig. 2: Variability of chlorophyll 'b' content (µg ml^{-1}) of different *T. arjuna* accessions as a function of non-stress and drought stress-controlled environment conditions. A1:718, A2:135, A3102, A4:115, A5-123, A6:614, A7-525, A8-507, A9-523, A10-424.

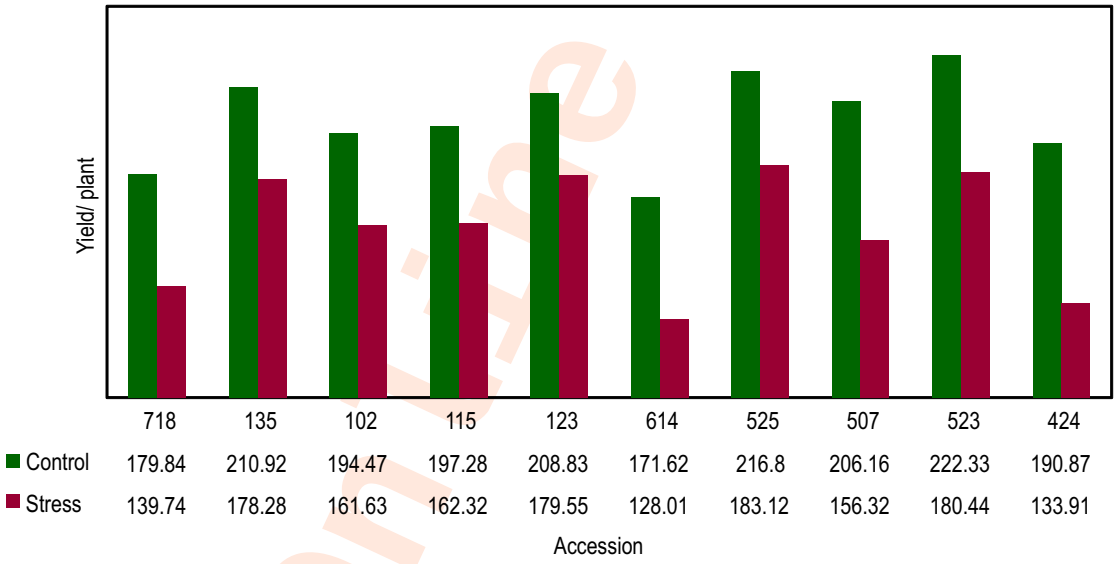

Fig. 3: Variability of leaf yield (g $plant^{-1}$) of different *T. arjuna* accessions as a function of non-stress and drought stress under controlled environment conditions.

cultivars' capacity for drought tolerance (Huang *et al.,* 2020). The proline levels of *T. arjuna* under control condition were highest with Accn 525, followed by Accn 523 and 135 (which were all significantly similar to each other), and lowest in Accn 614, followed by Accn 718 throughout the period (Table 3). Water stress increased proline concentrations in all accessions of *T. arjuna*, reaching 3–16 fold over the non-stressed control. Also, among different accession of *T. arjuna* under stress environments, the proline level significantly increased in Accn 525, followed by 523 and 135 at 7 and 14 DAT. However, during the initial period, Accn. 523 was observed with higher proline content, but it was on par with Accn. 525. Several studies have indicated that proline content increases during drought stress and that proline accumulation is associated with drought improvement. Accumulated proline is thought to participate in stress-protective functions as antioxidants and stabilisation of

macromolecules during drought (Seki *et al.,* 2007).

The results of this study confirm the reports (Alves and Setter (2004) that there is an increase in the proline concentration in water-stressed plants. In Accn. 525, 523 and 135 of the Arjuna plant, the increase in proline concentration might be due to more rapid biosynthesis of proline from its precursors. The host plants are able to support the photosynthetic apparatus by maintaining elevated organ hydration and turgor pressure due to accumulated proline, which serves to minimise osmotic potential and, in turn, leaf water potential (Kandowangko *et al.,* 2009).

The Arjuna accessions exhibited large differences in their responses to withholding water. Although the duration of the withholding of water was short, the adverse effects were actually severe under stress conditions. The moisture regime significantly affected the leaf yield of Arjuna plant. The data on leaf yield revealed that leaf yield under control conditions ranged from 171.62 to 222.34 g per plant, with a mean value of 199.91 g per plant (Fig. 3). The highest leaf yield of Arjuna plant was recorded by Accn 523 (222.34 g per plant), followed by Accn 525 (216.81 g per plant) and 135 (210.92 g per plant). Accn 614 with 171.62 g per plant showed the lowest leaf yield per plant under control conditions. Further, Accn 523 produced 29.56% more leaf yield as compared to Accn 614. Similarly, Accn 525 and 135 were 26.33 and 22.90% more leaf yield than Accn 614. Likewise, under moisture stress condition, it ranged from 128.01 to 183.13 g per plant with a mean value of 160.33. Higher leaf yield was recorded by Accn 525 (183.13 g per plant) and 523 (180.44 g per plant) and 123 (179.55 g per plant) and they all did not differ statistically. Accn 614 and 424 showed the lower leaf yield per plant. Accn 525, 523, and 123 produced 43.06, 40.96, and 40.26% more leaf yield per plant than Accn. 614. Therefore, in the present investigation, all the accessions showed a reduction in leaf yield under drought stress conditions as compared to control.

The reduction in leaf mean values in the yield trait is probably due to long period of stress experienced during the developmental stage. Drought disrupts growth, relationship between nutrients and water, photosynthesis, assimilate partitioning, and finally results in a major decrease in agricultural production (Praba *et al.,* 2009; Dencic *et al.*, 2000). Earl and Davis, (2003) reported that the primary yield-reducing factors under conditions of low soil moisture supply include reduced absorption of photosynthetically active radiation, poor radiation use efficiency, and low harvest index. Under water-limiting conditions, the plant height, leaf size and stem girth decreased dramatically in maize (Khan *et al.,* 2015). However, a few accessions, *i.e.,* Accn 525, 135 and 123, showed very low leaf yield reduction under stress conditions.

Such accessions can be used to study the mechanisms involved in drought tolerance. In comparison with the control, a few accessions showed better performance for leaf yield than moisture-sensitive accessions under water stress conditions. The above accessions were found superior to Accn 614 with respect to leaf yield under drought-stress conditions. The higher leaf yield of the above accessions was due to the maintenance of cell water turgidity and regulation of osmolytes up to a certain extent in drought-stress conditions. Plants alter their growth patterns and physiological processes to adapt to severe consequences of drought stress (Duan *et al.,* 2007).

It can be concluded that these accessions can be intended for further evaluation to develop drought-tolerant cultivars for block plantations in drought-affected areas. Based on the biochemical traits, higher chlorophyll and proline contents were found with Accn 523, 525, 135 of Arjuna plant reflecting its higher capability to tolerate drought stress.

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Authors' contribution: T. Pandiaraj: Conceptualization and designing of the experiments; collection of soil samples from study regions, lab experiments; **H. Yadav:** Conceptualization and designing of the experiments; Preparation of manuscript; **Manjappa:** Technical support in laboratory work; Preparation of manuscript; **J. Singh:** Preparation of manuscript, interpretation of results; **K. Sathyanarayana:** Support in execution of work; **D.K. Singh:** Preparation of manuscript; **P. Yadav:** Preparation of manuscript.

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Conflict of interest: The authors declare that is there is no conflict of interest.

Data availability: Available with the PI.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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