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***RESEARCH PAPER***

# Proline, chlorophyll stability index and relative water content interpretation of water stress adoption in Kodo millet (*Paspalum scrobiculatum*) genotypes

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**Abstract :** Kodo millet (*Paspalum scrobiculatum* L.) is one of the important nutricereal crops, which is mainly cultivated in India. Water stress is considered the most devastating environmental stress, which decreases crop productivity more than any other environmental stress. Drought is the most important abiotic factor considered as one of the crop performances limiting factors and a threat for successful crop production. The aim of this study was to evaluate and compare water stress effects on chlorophyll content, relative water content, chlorophyll stability index, proline and yield of varagu genotypes, as well as reveal which genotypes better adopts to water stress conditions using these parameters. From this assessment the most reliable parameter for drought tolerant, it is evident that the chlorophyll stability index (90 %), relative water content (88 %) and proline (980) is high in TNPsc 176 varagu genotype than the other genotypes.

**Key Words :** Chlorophyll content, Relative water content, Chlorophyll stability index, Proline yield, Water stress

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## INTRODUCTION

Millets represent small grain crops that are mainly cultivated in marginal environments.Millets are resilient to the extreme climatic and soil conditions. The similarities of millets are that they are all grown under extreme environmental conditions, especially those of inadequate moisture and poor soil fertility which are poorly suited to the major crops of the world (Baker, 2003). The ability for water or plant water use efficiency is greater, will be more resistant to drought and adaptation of some plants, such as plants CAM and C4 is that their metabolic pathway, allow them to exploitation of the dry environments, as well as the mechanisms of adaptation of plants that are activated in response to water stress (Kafi and Mahdavi Damghani, 1999). Millets possess a C4 photosynthesis system; hence, they prevent photorespiration and, as a consequence, efficiently utilize the scarce moisture present in the semi-arid regions (Brutnell, 2010, Warner and Edward, 1988). Since C4

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plants are able to close their stomata for long periods, they can significantly reduce moisture loss through the leaves.

Chlorophyll is one of the major chloroplast components for photosynthesis (Rahdari *et al.*, 2012). The decrease in chlorophyll content under drought stress has been considered a typical symptom of pigment photo oxidation and chlorophyll degradation (Anjum *et al.*, 2011). Decreased of chlorophyll content during drought stress depending on the duration and severity of drought level (Zhang and Kirkham, 1996). A decrease of total chlorophyll content with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Mafakheri *et al.*, 2010). In relation to drought effect on chlorophyll a and b in leaf, we can express that drought is due to chloroplastic proteins hydrolysis, decreasing of leaf pigments and chlorophyll destruction as a primary stagein degradation of proteins (Synerri *et al.*, 1993). At the onset of water stress, inhibition of cell growth, leading to a reduction in leaf development. Lower leaf surface causes less water uptake from the soil and transpiration is reduced. Plenty of water to form effective for a longer period, the soil is kept. Restrictions on the leaf surface could be the first line of defence against water (Kafi and Mahdavi Damghani, 1999). Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil moisture, whereas drought tolerance is the ability to withstand water deficit with low tissue water potential.

A study by Winkel *et al.,* 1997 in Niger where the annual rainfall is around 200 mm investigated the impact of water deficit at three stages of pearl millet development. The three stages were prior to flowering, at flowering and at the end of flowering. According to the findings of the work, the grain yield of pearl millet was severely reduced when moisture was limited, abiotic and biotic stress in plants recent advances and future perspectives and at the flowering stage but not at the end of flowering. On the other hand, in pearl millet, terminal drought in which irrigation was terminated from the flowering until crop maturity, was severe, as it resulted in 60% yield loss (Bidinger and Mahalakshmi,1987). The mid-season stress, which occurred from one month before flower initiation to full flowering, resulted in only 7% yield loss. An artificially created water stress environment will be used to provide the opportunity in selecting superior genotype out of a large population.

Tolerance to abiotic stresses is very complex, due to intricate of interaction between stress factors and various molecular, biochemical and physiological phenomenon affecting plant growth and development (Razmjoo *et al.*, 2008). High yield potential under drought stress is the target of crop breeding. In many cases, high yield potential can contribute to yield in moderate stress environment (Blum,1996). In plants, a better understanding of the morpho-anatomical and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions (Martinez *et al.*, 2007).

The best characterized biochemical response of plant cells to osmotic stress is the accumulation of organic osmolytes, most commonly proline and betaines (Mc Cue and Hanson, 1990). The leaf of tolerant genotype maintained higher RWC, Photosynthetic activity and proline levels under water stress than that of drought sensitive genotype (Asishkumar, 2008).The synthesis of proline helps the cell to maintain their hydrated state and therefore functions to provide resistance against drought and cellular hydration (Ramanjulu and Bartels, 2002).

Millets play a significant role in the livelihood of the population of developing world especially due to their enormous contribution to the food security of all countries. However, these crops have not been sufficiently studied. Breeding for drought tolerance is the major objective of many crop-breeding programmes due to the widespread prevalence of the moisture-deficit problem in global agriculture. A number of crops with drought tolerance have been developed. There are two options for the management of crops in water-limiting environments: the genetic and agronomic (Saxena and John, 2002). The genetic approach requires robust and reproducible screening methods for the identification of traits of drought tolerance in germplasm and breeding materials, and incorporation of the same into high-yielding varieties using conventional and biotechnological tools.

## MATERIAL AND METHODS

A field experiment was conducted at Centre of Excellence in millets, Athiyandal, Tiruvannamalai district, Tamil Nadu, India. Randomized Block Design was used to conduct this experiment with four replications. Five varieties of *Paspalum scorbiculatum* L., CO 3, TNAU

86, TNPsc 176, TNPsc 301 and TNPsc 313 were used in this study. Terminal stress was imposed at flower initiation period of crop growth. Duration of the crop was 120 days. Varagusown with a spacing of 45 cm x 10 cm and raised with recommended package of practices.

**Chlorophyll content :**

Contents of fractions of 'a', 'b' and total chlorophyll were estimated in a fully expanded young leaf at the specified time intervals and expressed in mg $g^{-1}$ fresh weight (Yoshida *et al.,* 1971).

**Relative water content (RWC) :**

Leaf samples were taken from the youngest fully expanded leaves for recording relative water content. Relative water content was estimated by Weatherly (1962) method and expressed in percentage.

**Chlorophyll stability index (CSI) :**

Chlorophyll stability index was calculated by the method described by Murthy and Majumdhar (1962).

**Proline content :**

Proline content of leaves was estimated by the method described by Bates *et al*. (1973) and expressed in µ moles $g^{-1}$ fresh weight.

## Results and Discussion

In the present study, there was significant reduction in growth expressed in plant height irrespective of genotypes, treatments and their interaction. Drought stress imposed at pre flowering stage as more pronounced effect on plant height than at any other stages. The plant height (Table 1) recorded at pre flowering stage was reduced in the moisture stress-imposed treatment from 96 to 85 cm the reduced plant stature under moisture deficit stress are similar to that reported by Gerik *et al*. (1996) and Ball *et al.* (1994). The genotype TNPsc 176 has the highest value (85.7 cm) than other genotypes at grain filling stage. Root elongation during drought may help plants get deeper water, thus avoiding water deficits near the soil surface. Elongation also could reduce the water lost by drainage when precipitation allows recovery after the drought (Ludlow and Muchow, 1990). If, however, water is unavailable deeper in the soil profile, longer roots may reduce shoot dry weight and harvest index by allowing the preferential partitioning of photosynthate to roots at the expense of shoots. The genotype TNPsc 176 has increased root length (22.3) significantly at grain filling stage than other genotypes. Data on leaf area revealed significant differences between the genotypes. The genotype TNPsc 176 maintains a higher mean leaf area during grain filling stage than others even under the stress condition (453.8). The genotype CO3 has the lowest value 397.3 than other genotypes at all stages irrespective of the treatment effects.

Hussain *et al*. (2009) reported decline in LAI of sunflower exposed to drought at budding and flowering stages. Drought also suppresses leaf expansion and tillering (Kramer and Boyer, 1995), and reduces leaf area due to early senescence (Nooden, 1988). All these factors contribute to reduced dry matter accumulation and grain yield under drought. In pearl millet (*Pennisetum glaucum* L. Leeke), drought at flowering increased the rate of ear abortion due to a decline in assimilate supply to developing ears (Yadav *et al*., 2004). In drought-stressed maize, kernel set was lost leading to low grain yield (Schussler and Westgate, 1995). Likewise, water deficit at anthesis increased pod abortion which reduced yield in soybean (Liu *et al.,* 2003). The role of photosynthetic

**Table 1: Effect of drought on plant height (cm), leaf area ($cm^2$ $plant^{-1}$), leaf area index, specific leaf weight (mg $cm^2$) specific leaf area ($cm^2$ $g^{-1}$) total dry matter production (g/ $cm^2$) and crop growth rate (g $m^{-2}$ $day^{-1}$), of varagu genotypes**

| Genotypes | Plant height (cm) | Leaf area ($cm^2$ $plant^{-1}$) | Leaf area index | Specific leaf weight (mg $cm^2$) | Specific leaf area ($cm^2$ $g^{-1}$) | TDMP (g/ $cm^{2)}$ | Crop growth rate (g $m^{-2}$ $day^{-1}$) |
|---|---|---|---|---|---|---|---|
| CO 3 | 81.3 | 397.3 | 0.883 | 6.823 | 146.57 | 39.29 | 0.452 |
| TNAU 86 | 74.3 | 412.4 | 0.916 | 4.135 | 140.15 | 42.16 | 0.438 |
| TNPsc 176 | 85.7 | 453.8 | 1.008 | 7.761 | 173.59 | 46.27 | 0.564 |
| TNPsc 301 | 76.0 | 421.3 | 0.936 | 6.254 | 159.89 | 38.92 | 0.433 |
| TNPsc 313 | 79.3 | 424.5 | 0.943 | 5.95 | 125.78 | 43.41 | 0.534 |
| S.E.± | 1.46 | 2.15 | 0.02 | 0.56 | 0.82 | 0.45 | 0.04 |
| C.D. (P=0.05) | 4.52 | 6.63 | 0.05 | 1.02 | 1.64 | 1.02 | 0.08 |

pigments such as chlorophyll (chl) contents, carotenoids, and xanthophylls are also vital in carbon fixation, as they are involved in capturing solar radiation to drive the photosynthetic mechanism. Drought stress severely decreased chla andchl b contents in marigold (Asrar and Elhindi, 2011).

Drought lowered RWC in tomato and caper bush (*Capparis spinosa* L.) (Ozkur *et al.,* 2009). In sunflower, RWC, leaf water potential and osmotic potential were affected by drought (Tezara *et al.*, 2002). However, different genotypes behaved differently; drought-tolerant genotypes maintained higher leaf water potential for longer and wilted later than sensitive genotypes upon exposure to drought (Ouvrard *et al.,* 1996). Breeding approach is often used to explore genetic variability for drought tolerance among crop genotypes for desired agronomic traits to then breed genotypes better able to perform in drought-prone areas (Ashraf, 2010).

Water stress inhibits cell enlargement and reduce plant growth by affecting various physiological and biochemical processes, such as leaf area, chlorophyll content and photosynthesis.The genotype TNPsc176 maintained higher total chlorophyll content in all stages than others even under the stress conditions (2.572). The total chlorophyll values were significantly lower in the case of TNPsc 301(2.074). The chlorophyll stability index is an indicative of the maintenance of photosynthetic pigments under drought situation. Among the genotypes the TNPsc 176 and TNPsc 313 maintained a good mean values (84% and 82%). Among the genotypes TNPsc176 has recorded the highest proline content at grain filling stage (980) than other genotypes (Fig. 1). The number of productive tillers and no of grains per panicle were found to be highest in TNPsc 176 followed by TNPsc 313 and TNAU 86, genotypes.The stress imposed at grain filling stage has shown a marked reduction in seed cotton yield in all the genotypes. The genotype TNPsc 176 have the highest value of grain yield (1782) than other genotypes (Table 2).

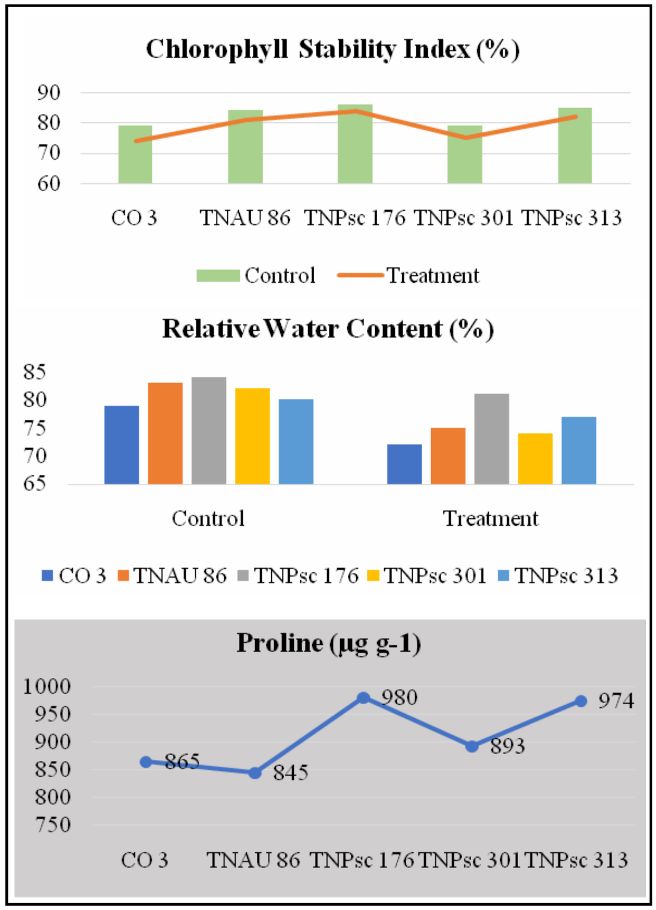


**Fig. 1: Effect of drought on chlorophyll stability index (%), Relative water content (%) and proline (µg $g^{-1}$) of varagu genotypes at grain filling stage**

## Conclusion:

Using plant breeding is a good approach for improving drought tolerance, also, produce appropriate drought tolerant genotypes could be another technique

**Table 2 : Effect of drought on total chlorophyll content (mg/g), proline content (µg $g^{-1}$), yield and yield components of Varagu genotypes**

| Genotypes | Total chlorophyll content (mg/g) | Proline content (µg $g^{-1}$) | No of productive tillers / hill | Days to 50% flowering | No of grains per panicle | 1000 grain weight | Yield (kg/ ha) |
|---|---|---|---|---|---|---|---|
| CO 3 | 2.127 | 865 | 10 | 54 | 44.67 | 5.89 | 1460 |
| TNAU 86 | 2.329 | 845 | 13 | 54 | 40.67 | 5.09 | 1325 |
| TNPsc 176 | 2.572 | 980 | 17 | 48 | 56.60 | 5.96 | 1782 |
| TNPsc 301 | 2.094 | 893 | 16 | 52 | 36.00 | 5.37 | 1561 |
| TNPsc 313 | 2.457 | 974 | 14 | 51 | 51.67 | 5.55 | 1680 |
| S.E.± | 0.03 | 2.54 | 0.62 | 1.46 | 2.59 | 0.10 | 42 |
| C.D. (P=0.05) | 0.09 | 5.28 | 1.34 | 4.51 | 7.99 | 0.30 | 118 |

to manage drought stress and improve plant response to stresses.Plants acclimatize with drought stress through use various strategies which include drought escape, drought avoidance and drought tolerance.

**Future scope:**

Drought stress is one of the most serious threats to world food security. The Proline, Chlorophyll Stability Index and Relative Water Content were not analyzed for most of the minor millet in India. Physiology and classical breeding techniques for improving plant drought tolerance, could improve plant tolerant for drought stress.

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