**International Journal of Ecology and Environmental Sciences**
www.ecologyjournal.in
Online ISSN: 2664-7133; Print ISSN: 2664-7125; Impact Factor: RJIF 5.28
Received: 26-12-2020; Accepted: 10-01-2021; Published: 29-01-2021
Volume 3; Issue 1; 2021; Page No. 204-208

# Effect of moisture stress on yield, quality and biochemical attributes of chia (*Salvia hispanica* l.) genotypes

**Shivashankar[1*], Sadashiv Nadukeri[1], Raviraja G Shetty[1], Ganapathi M[1], Shashikala Kolakar[1], Sreedhar RV[2]**
[1] College of Horticulture, Mudigere, Karnataka, India
[2] Central Food Technological Research Institute, Mysore, Karnataka, India

**Abstract**
A pot experiment was conducted at College of Horticulture, Mudigere under protected condition to study the effect of moisture stress on performance of four local genotypes of chia *viz.*, H. D. Kote local, Mysore local, T. Narasipura local and Nanjangud local with three moisture stress levels *i. e.* 50, 75 and 100 % (Control) of field capacity. The experiment was laid out in a factorial completely randomized design with three replications. The results of the study inferred that different genotypes showed variable results on different attributes due to varying physiological constraints. Genotype H. D. Kote local showed better performance concerning yield, quality and biochemical attributes. While, the genotype Mysore local was found inferior. Severe water stress (50 % of FC) reduced the number of spikes per plant, seed yield per plant (g/plant) and per hectare (kg/ha), test weight of seeds (g), drought tolerance index and protein content (%). However, moderate stress (75 % of FC) registered fewer days to fifty per cent flowering and favored higher protein content in seeds. Likewise, severe stress favoured higher proline content (µg/g fresh weight) in leaves. Therefore, control (100 % of FC) was found beneficial for higher seed yield in chia genotypes.

**Keywords:** water stress, field capacity, genotype, seed yield, protein, proline

## Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous oilseed plant belonging to the Lamiaceae family, which is native from Southern Mexico and Northern Guatemala. It has been cultivated from tropical to subtropical regions. Chia is a dicotyledonous plant, approximately a meter tall, with opposite, petiolate and serrated leaves that are 4 to 8 cm long and 3 to 5 cm wide. The plant has ribbed and hairy quadrangular stems. The flowers are hermaphrodite and occur in numerous clusters in a spike protected by small bracts with long pointed tips. The fruit of chia is a schizocarp comprising of indehiscent locules that separate to form four fruitlets, referred to mericarps or nutlets. Commercially, each of these fruitlets is called ‘seed’, but actually, the (true) seed is contained within each fruitlet. The seeds are oval, smooth, shiny and their colour varies from black, gray and black spotted to white and size ranges from 1 mm to 2 mm (Ayerza and Coates, 2005) [4]. Chia crop is recognized as a superfood crop for its superior nutritional value. The seed has about 25-38 per cent oil by weight and it contains the highest proportion of α-linolenic acid (60 %) compared to other natural source known to date (Ayerza, 1995) [3] and also higher levels of protein (19-23 %) in addition to other important nutritional components, such as vitamins, minerals and natural antioxidants (Coates and Ayerza, 1996) [7].

India, which also remains high under the threat of malnutrition, has scarce commercial cultivation of chia crop and its consumption as well. Chia seeds are currently imported from countries like Australia, Bolivia and the US, which are sold at the rate of Rs. 2000 per kg in India. Currently, chia seed offers a huge potential in the industries of health, food, animal feed, pharmaceuticals, and nutraceuticals, due to its functional components (Mary, 2017) [16]. Though it is an introduced crop, area under cultivation of this crop is also gradually increasing in India while offering a potential and bright future to the Indian farmers and agro-markets. Studies on agronomic management of chia crop are limited as it is a newly introduced crop to India and Karnataka in particular. In recent years, the cultivation of this crop has been started in Karnataka by the farmers of Mysore and Chamrajanagara districts under the technical guidance of the Central Food Technological Research Institute (CFTRI), Mysore. In the changing climate scenario, plants are continuously subjected to several biotic and abiotic stresses. Among these stresses, drought is one of the most severe abiotic stress which threatens crops production and yield. Crops undergo various morphological, physiological, biochemical, and molecular responses under water stress. Drought or water-limited situation triggers a wide variety of plant responses, ranging from cellular metabolism to changes in growth and development of roots, shoots, and ultimately yield.

Further, in recent times, water has become a scarce resource due to erratic rainfall and depleting groundwater resources in India as well as in Karnataka. Water stress is the main limiting environmental factor in most of the areas in the world (Valliyodan and Nguyen, 2006) [20], and various investigations have been performed in several plant species. *Salvia hispanica* L. has been described as a species highly tolerant to water deficit, but in ecological-adaptive responses to water deficits are unknown (Silva *et al’’*., 2018). As reports suggest that chia fairly responds to drought, the effect of different levels of moisture stress on the performance of this crop needs to be studied. So, the present investigation was planned to investigate the genetic potentiality of four local chia genotypes across three moisture stress levels.

## Material and Methods
### Experimental details
A pot experiment was carried out in the polyhouse of the Medicinal plants block, College of Horticulture, Mudigere. The study area lies between 130 08' to 130 53' Northern latitude and between 750 04' to 760 21' Eastern longitude and located at an altitude of 982 m above mean sea level. The experiment was laid out in a factorial completely randomized design with three replications. Treatment combinations were four genotypes *viz*., H. D. Kote local ($V_1$), Mysore local ($V_2$), T. Narasipura local ($V_3$) and Nanjangud local ($V_4$) with three levels of moisture stress *viz*., 50 % ($S_1$), 75 % ($S_2$) and 100 % ($S_3$ - Control) of field capacity. Thus, there were twelve treatment combinations *viz*., $T_1 = V_1S_1$, $T_2 = V_1S_2$, $T_3 = V_1S_3$, $T_4 = V_2S_1$, $T_5 = V_2S_2$, $T_6 = V_2S_3$, $T_7 = V_3S_1$, $T_8 = V_3S_2$, $T_9 = V_3S_3$, $T_{10} = V_4S_1$, $T_{11} = V_4S_2$ and $T_{12} = V_4S_3$.

### Plant material and growing conditions
The seeds of chia genotypes were collected from respective localities within Mysore district of Karnataka. Seedlings were raised in the portrays with plugs filled with sterilized coco peat. The seedlings were ready for transplanting in 20 days from sowing. In the experiment, 288 plastic pots of 22 litres capacity and 32 cm depth were used. Red soil was sterilized with formalin and covered with black polythene cover for ten days. Soil, sand and FYM (Farm yard manure) were mixed in 2:1:1 ratio and filled in pot.

Three seedlings of 20 days old were transplanted to pots as per the treatment and plan of layout. Further, seedlings were immediately irrigated and drenched with bavistin at 0.2 per cent to avoid fungal diseases. Thinning was done at 12 days after transplanting retaining one plant per pot, so as to maintain optimum plant population. Irrigation was provided based on the condition of soil and crop, mainly at an interval of 3-4 days during the establishment period and later the interval was increased to 6-7 days up to 20 days of transplanting until all the seedlings reached 6 leaf stage. Moisture stress treatment was imposed to plants at 20 days after transplanting seedlings in pots. Weeding was done as and when necessary. All the plants of the given genotypes were harvested at 90 days after transplanting.

### Measurements
Days to 50 per cent flowering, number of spikes per plant, seed yield per plant (g/plant) and per hectare (kg/ha), test weight of seeds (g), drought tolerance index, protein content (%) and proline content (μg/g fresh weight) were recorded.

### Yield and yield parameters
### Days to 50 per cent flowering
The number of days taken for 50 per cent flowering from the date of transplanting was recorded in plants of each treatment.

### Number of spikes per plant
The total number of spikes per plant was counted for each selected plants.

### Seed yield per plant (g/plant)
Seeds harvested from each selected plant of each treatment were weighed on electronic balance and the average was computed and thus the seed yield was expressed in grams per plant.

### Seed yield per hectare (kg/ha)
Seed yield per hectare was calculated by multiplying the yield per plant with the number of plants per hectare (81,632) and the mean yield per hectare was worked out.

### Test weight of seeds (g)
The test weight of seeds was recorded by weighing 1000 seeds from each treatment.

### Drought tolerance index
The drought tolerance index was calculated by using the formula of Fernandez (1992).

$$DTI = (Yp \times Ys)/(Yx)^2$$

Where,
Yp = Grain yield of each treatment under non-stress condition
Ys = Grain yield of each treatment under stress condition
Yx = Mean grain yield of all treatments in non-stress condition

### Quality and biochemical parameters
### Protein content (%)
Seeds and defatted meals were powdered using mortar and pestle and used for the estimation of protein. Crude nitrogen of the chia seeds was determined by alkaline permanganate method using the Kjeldahl distillation unit and then converted to protein content using a 5.71 conversion factor (AOAC, 1997) [2].

### Proline content (μg/g fresh weight)
1 gram of fresh leaf sample was homogenized in 10 ml of 3 per cent sulphosalicylic acid and the homogenate was filtered. 2 ml of filtrate was taken and to that 2 ml of glacial acetic acid and 2 ml of acid ninhydrin were added (Acid ninhydrin: 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M orthophosphoric acid). This was heated in a boiling water bath for an hour. Placing the test tubes in an ice bath terminated the reaction, 4 ml of toluene was added and stirred well. The toluene layer was separated using a separating funnel and warmed to room temperature. The intensity of red colour was measure at 520 nm. The amount of proline in the sample was calculated using a standard curve prepared from pure proline (range 0.1 to 36 μmole) and expressed on a fresh weight basis of sample (Bates *et al''*., 1973) [5].

$$\text{Proline } (\mu g/g \text{ fresh weight}) = (\mu g \text{ Proline/ml} \times \text{ml Toluene})/115.5 \times 5/g \text{ sample}$$

Where, 115.5 is the molecular weight of proline.

### Statistical analysis
The data on morphological and physiological parameters collected from the experiment were subjected to statistical analysis by adopting Fisher's method of analysis of variance as outlined by Gomez and Gomez (1984). The level of significance used in the 'F' test was at 5 per cent. The critical difference (CD) values are given at 5 per cent level of significance, wherever the 'F' test was significant.

## Results and Discussion
Different genotypes showed variable results on different attributes due to varying physiological constraints. Water stress caused a significant decrease in the number of spikes per plant, seed yield per plant (g/plant) and per hectare (kg/ha), test weight

of seeds (g), drought tolerance index and protein content (%) (Figure 1 and Table 1). While, moderate stress registered fewer days to fifty per cent flowering and favored higher protein content in seeds (Table 1). Likewise, severe stress favoured higher proline content (µg/g fresh weight) in leaves (Figure 2).

The genotype H. D. Kote local under moderate stress (75 % of FC) produced fifty per cent of flowers in fewer days (59.89). While, the genotype Mysore local under severe stress (50 % of FC) had taken maximum number of days (64.33) to produce fifty per cent of flowers. However, the combined effect of genotypes and moisture stress on days to 50 per cent flowering remained non-significant (Table 1). Early flowering is a desirable trait as it provides sufficient time for grain formation and filling. The outstanding example is proceeding to the flowering phase when water is limited; basically, the plant shortens the life cycle in this way. In this strategy the plant does not invest too much in up-regulating the metabolic pathways against stress, instead, it maximizes the pathways for fast growth to pass the moderate crisis (Verslues and Juenger, 2011) [21].

The highest number of spikes per plant (20.16) was observed in genotype H. D. Kote local under control (100 % of FC) condition which was on par with the genotype H. D. Kote local under 75 per cent of field capacity (19.11) and the genotype Nanjangud local under control (19.05). While, the lowest number of spikes per plant (11.50) was observed in genotype Mysore local under severe stress (50 % of FC) condition (Figure 1). Similar observations were reported by Tardieu (2012) [19] in wheat. The decrease in the number of spikes under water stress could be attributed to different stress injury degrees and the destruction of sources and sink (Ding *et al''*., 2018) [8].

Significantly, the highest seed yield per plant (4.95 g/plant) (Figure 1) and seed yield per hectare (404.08 kg/ha) (Table 1) was noticed in the genotype H. D. Kote local under control. While, the lower seed yield per plant (1.94 g/plant) (Figure 1) and seed yield per hectare (158.37 kg/ha) (Table 1) was noticed in the genotype Mysore local under severe stress (50 % of FC). Similar observations were reported by Pourjavadian *et al''*. (2015) [17] in *Satureja hortens*. The negative impacts of drought on the yield mainly depend upon the severity of the stress and the plant growth stage. The apparent marked reduction in seed yield per plant could be attributed to restricted seed set caused by dehydration and reduced assimilate partitioning. Water stress reduces the source strength by reducing photosynthesis. Thus, decreasing the translocation of metabolites contributing to yield (Farooq *et al*., 2012) [10].

Data on 1000 seeds weight did not indicate significant variations. However, numerically higher 1000 seeds weight (1.25 g) was recorded in genotype H. D. Kote local under control. While, the lower test weight (1.13 g) was observed in genotype T. Narasipura local under severe stress (50 % of FC) (Table 1). The reason could be attributed to the fact that, chia seeds are very minute and the weight will not vary much due to treatment effects. The highest drought tolerance index (1.36) was recorded in the genotype H. D. Kote local under control and the least (0.32) was observed in genotype Mysore local under severe stress. However, the combined effect of genotypes and moisture stress on drought tolerance index remained non-significant (Table 1). Similar observations were reported by El-Hendawy *et al''*. (2017) [9] in wheat. The increased DTI under irrigated control over stressed condition could be attributed to increased seed yield and efficiency of individual plant to convert water used to grain as quoted by Jabereldar *et al''*., 2017 [15].

Higher protein content (23.35 %) was recorded in the genotype H. D. Kote local under moderate stress (75 % of FC) and the least (18.08 %) was recorded in genotype T. Narasipura local under severe stress (50 % of FC). However, the combined effect of genotypes and moisture stress on protein content in seeds remained non-significant (Table 1). Similar observations were reported by Hendawy and Khalid (2005) [13] in *Salvia officinalis* L. Moderate drought stress induces a large number of genes encoding for the biosynthesis of proteins. Increasing stress from mild to severe level, the plant reduces its ability to synthesize the protein (Ingram and Bartels, 1996) [14].

Significantly, the highest proline content (76.06 µg/g fresh weight) was recorded in the genotype H. D. Kote local under severe stress (50 % of FC) which was on par with the genotype Nanjangud local under severe stress (75.39 µg/g fresh weight). While, the least proline content (24.37 µg/g fresh weight) was noticed in genotype Mysore local under control (Figure 2). Similar observations were reported by Al-Ghamdi (2019) [1] in marjoram. Proline is an index of drought resistance and its accumulation contributes to maintain proper balance between extracellular and intracellular osmolarity under water stress. The increased concentration of proline in response to drought is due to decreased leaf relative water content, increased proteolysis, and decreased protein synthesis (Blum and Ebercon, 1976) [6].

**Table 1:** Effect of moisture stress on yield and quality attributes in chia (*S. hispanica* L.) genotypes

| Genotype | Moisture stress | Days to 50 per cent flowering | Seed yield per hectare (kg/ha) | Test weight of seeds (g) | Drought tolerance index | Protein content (%) |
|---|---|---|---|---|---|---|
| H. D. Kote local | 50 % of FC | 63.08 | 248.16 | 1.19 | 0.83 | 19.26 |
| | 75 % of FC | 59.89 | 321.90 | 1.21 | 1.08 | 23.35 |
| | 100 % of FC | 61.33 | 404.08 | 1.25 | 1.36 | 21.26 |
| Mysore local | 50 % of FC | 64.33 | 158.37 | 1.15 | 0.32 | 18.13 |
| | 75 % of FC | 61.44 | 206.12 | 1.17 | 0.42 | 21.60 |
| | 100 % of FC | 62.33 | 244.08 | 1.18 | 0.49 | 20.32 |
| T. Narasipura local | 50 % of FC | 62.95 | 218.64 | 1.13 | 0.65 | 18.08 |
| | 75 % of FC | 60.93 | 247.07 | 1.15 | 0.74 | 22.06 |
| | 100 % of FC | 61.41 | 359.18 | 1.18 | 1.07 | 20.40 |
| Nanjangud local | 50 % of FC | 62.71 | 264.08 | 1.19 | 0.83 | 19.16 |
| | 75 % of FC | 60.33 | 300.68 | 1.20 | 0.94 | 23.11 |
| | 100 % of FC | 61.80 | 377.14 | 1.21 | 1.19 | 21.31 |
| SEm ± | | 1.54 | 8.62 | 0.04 | 0.05 | 0.24 |
| CD (P=0.05) | | NS | 25.88 | NS | NS | NS |

FC = Field capacity NS = Non-significant

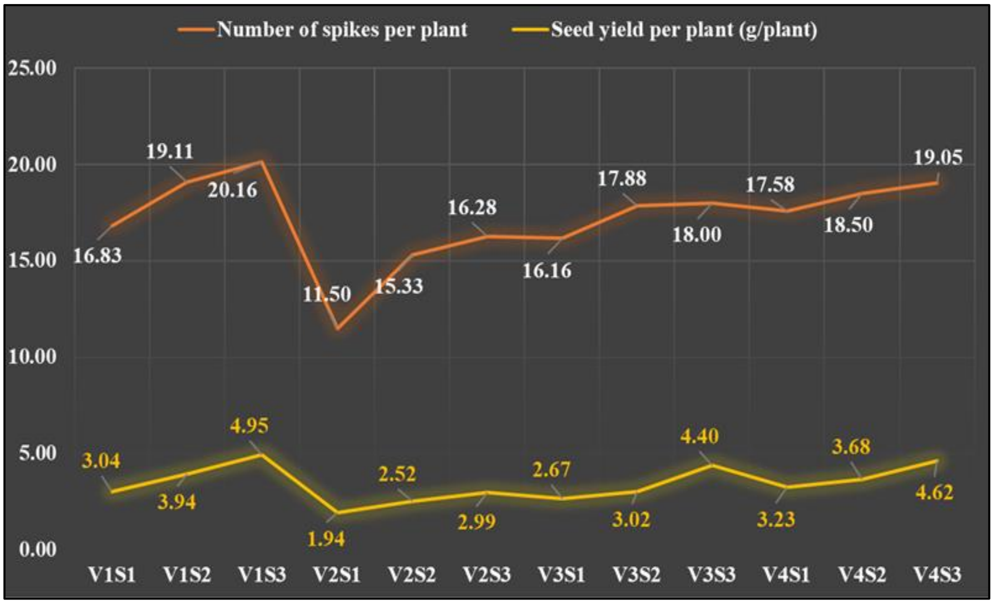


**Fig 1:** Effect of moisture stress on number of spikes and seed yield per plant in chia (*S. hispanica* L.) genotypes

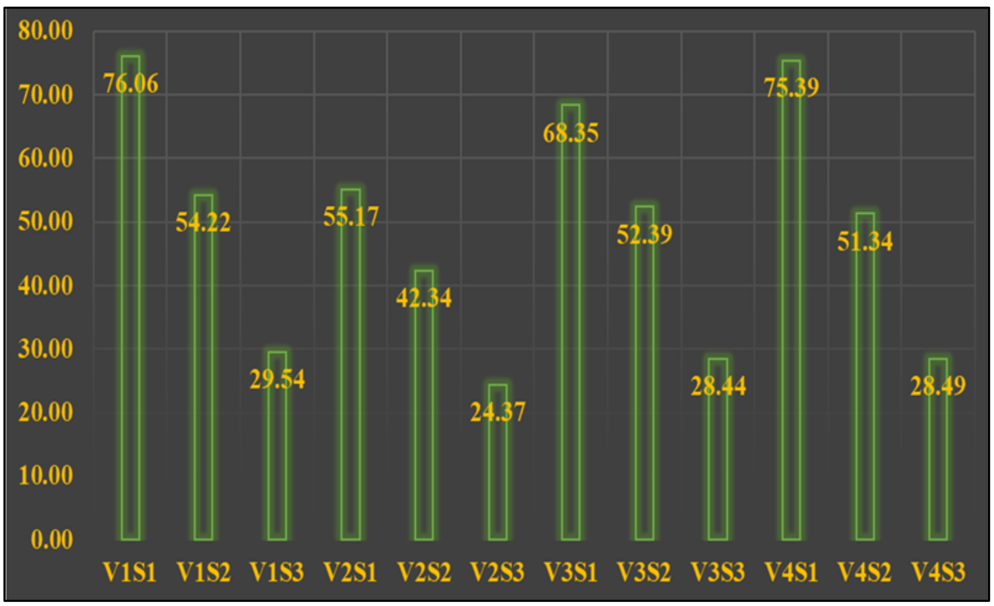


**Fig 2:** Effect of moisture stress on proline content in chia (*S. hispanica* L.) genotypes

## Conclusion

The results of the study inferred that different genotypes showed variable results on different attributes due to varying physiological constraints. Genotype H. D. Kote local showed better performance concerning yield, quality and biochemical attributes. While, the genotype Mysore local was found inferior. Severe water stress (50 % of FC) reduced the number of spikes per plant, seed yield per plant (g/plant) and per hectare (kg/ha), test weight of seeds (g), drought tolerance index and protein content (%). However, moderate stress (75 % of FC) registered fewer days to fifty per cent flowering and favored higher protein content in seeds. Likewise, severe stress favoured higher proline content (µg/g fresh weight) in leaves. Therefore, it was concluded that, interaction of genotype H. D. Kote local and control (100 % of FC) was found beneficial for higher seed yield in chia genotypes.

## Acknowledgements

The authors are highly acknowledged to University of Agricultural and Horticultural Sciences, Shivamogga, for providing all the support required for this research work.

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