Scientific Notes

Proline content of sunflower cultivars in the Brazilian semiarid region

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Abstract – The objective of this work was to evaluate the proline content of sunflower (*Helianthus annuus*) cultivars, in the conditions of natural water deficit in the Brazilian semiarid region. Thirteen cultivars were sown in experiments set in three sites in the Brazilian Northeast. Proline content was determined on leaves from plants at the R4–R5 stage. Significant differences among cultivars occurred only where water availability was lower. Proline averages ranged from 3.47 to 17.41 μg g^{-1}. The sunflower cultivars BRS387, BRS323, and BRS324 showed the greatest proline contents with 54.74, 46.27, and 35.16 μg g^{-1}, respectively. These are the cultivars that accumulate more proline under conditions of a severe water deficit.

Index terms: *Helianthus annuus*, drought, water deficit.

Teor de prolina de cultivares de girassol no Semiárido brasileiro

Resumo – O objetivo deste trabalho foi avaliar os teores de prolina em cultivares de girassol (*Helianthus annuus*), em condições de deficit hídrico natural na região do Semiárido brasileiro. Treze cultivares foram semeadas em experimentos estabelecidos em três áreas do Nordeste brasileiro. O teor de prolina foi determinado em folhas de plantas no estádio R4–R5. Diferenças significativas entre cultivares ocorreram apenas onde a disponibilidade de água foi menor. As médias de prolina variaram de 3,47 a 17,41 μg g^{-1}. As cultivares de girassol BRS387, BRS323 e BRS324 apresentaram os maiores teores de prolina com 54,74, 46,27 e 35,16 μg g^{-1}, respectivamente. Essas cultivares são as que acumulam mais prolina em condições de deficit hídrico severo.

Termos para indexação: *Helianthus annuus,* seca, deficiência hídrica.

The cultivation area of sunflower (*Helianthus annuus* L.) in Brazil is small; in the 2015/2016 harvest, it measured only 51.4 thousand hectares (Conab, 2017). However, as this oilseed has a great adaptation capacity to different latitudes, longitudes, and photoperiods, and it is more tolerant to drought than corn and sorghum, it has been considered an alternative crop for cultivation in the Brazilian semiarid region (Birck et al., 2017).

A number of studies regarding agronomic traits of sunflower are reported (Rauf, 2008); however, only few studies are conducted under drought stress (Casadebaig et al., 2008; Ghaffari et al., 2012; Pourmohammad et al., 2014; Khalil et al., 2016), and even less ones on drought under field conditions (Alza & Fernandez-Martinez, 1997). Most studies include only one or two cultivars. It is well known that sunflower yield decreases under drought stress, but this is dependent on the level of water deficit and cultivar used (Rauff, 2008).

The expansion of sunflower cultivation in the semiarid region is associated to the development and selection of cultivars adapted to this region and, consequently, with greater tolerance to water stress. This development can be effective by selecting genotypes with greater accumulation of compatible solutes as proline (Canavar et al., 2014). The proline accumulation might contribute, by lowering the cell osmotic potential to maintain turgescence (Cechin et al., 2010). Increases of proline levels have been determined under drought stress (Ghaffari et al., 2012; Canavar et al., 2014; Pourmohammad et al., 2014). In complement, Khalil et al. (2016) verified that genotypic variance and broad-sense heritability of proline concentration were high, particularly under osmotic stress, allowing of the use of this trait in breeding programs as one of the indicatives of tolerance to water deficit. These data can be useful in genotype selection (with higher-proline content), with potential to be used in crosses to obtain new strains and hybrids for the Brazilian semiarid region.

The objective of this work was to evaluate the proline content of sunflower cultivars, under natural water deficit in the Brazilian semiarid region.

Thirteen cultivars were assessed in a private farm in the semiarid regions (Table 1) of Frei Paulo (10°55'S, 37°53'W, at 272 m altitude) and Poço Redondo (9°47'S, 37°41'W, at 188 m altitude) municipalities, both in the state of Sergipe, Brazil, and in the municipality of Paripiranga (10°14'S 37°51'W, at 430 m altitude), in the state of Bahia, Brazil. Although the plantings were established during the rainy period, the accumulated rainfall was only 24.39 and 24.33 mm in Poço Redondo and Paripiranga, respectively, in contrast to the greater amount in Frei Paulo (283.9 mm). In Poço Redondo, in contrast to others, the experiment received a supplemental irrigation, early in the morning, during 40 min for five days a week, by a microsprinkler (Santeno, 7 L m^{-2} per day). Minimum, mean, and maximum air temperatures are shown on Figure 1.

The cultivars assessed were M 734 (Dow Agrosciences Industrial, São Paulo, SP, Brazil), CF 101 and Olisun 3 (Advanta Comércio de Sementes Ltda., Campinas, SP, Brazil), Helio 250 and Helio 251 (Heliagro Agricultura e Pecuária Ltda., Araguari, MG, Brazil), Aguará 4, Aguará 6 (Atlântica Sementes, Curitiba, PR, Brazil), BRS321, BRS322, BRS323, BRS387, BRS324, and Embrapa 122 (Embrapa, Brasília, DF, Brazil). Except for the last two cultivars (open-pollinated population), the others are hybrids and are registered in Brazil. They were chosen from the breeding program of Embrapa due their high yields. The experiments were set in a Cambisol (Santos et al., 2013), in a completely randomized block design, with four replicates. Each plot consisted of four rows of 6.0 m long, spaced at 0.70 m apart, with 0.30 m between holes, with a total of 47.667 plants ha^{-1}. The cultivars were sown by hand in July, at 3-inch soil depth. After planting and then emerging, plants were thinned, and only one plant was left in each hole. The recommended cultural practices were performed according to Oliveira & Rosa (2013).

The free-leaf proline content was measured according to Bates et al. (1973). Two fully expanded fresh leaves (3, 4, numbered acropetally) from four plants were collected in the midday, in September, at the beginning of flowering (R4–R5 stage), and immediately frozen at -20°C. The proline amount was estimated spectrophotometrically using ninhydrin (Bates et al., 1973). Purified proline (d-proline, Sigma-Aldrich, Saint Louis, MO, USA) was used for standardization. The proline content (μg g^{-1}) was expressed in fresh weight of each cultivar, and subjected to analyses of variance (F test), followed by a multiple comparison Scott Knott test, at 5 % probability.

There was a significant interaction between location and cultivar, which indicates that the differences between cultivars occurred as a function of location (Table 1). The cultivars showed 3.4720, 12.6264, and 17.4085 μg proline·g^{-1} of fresh weight, in Frei Paulo, Poço Redondo, and Paripiranga, respectively. The greatest means verified in the last two municipalities may be due to a more severe water stress in these locations, as the proline content in sunflower plants

Table 1. Variance analyses for proline content in fully expanded leaves from thirteen sunflower (*Helianthus annuus*) cultivars grown in the municipalities of Frei Paulo and Poço Redondo (Sergipe state), and in the municipality of Paripiranga (Bahia state), Brazilian semiarid region, in 2014[(1)].

Cultivar	Proline content (μg proline g^{-1} fresh weight)		
	Frei Paulo	Poço Redondo	Paripiranga
M 734	4,052.7aA	12,625.0aA	3,743.3bA
Embrapa122	2,301.7aB	13,265.3aA	19,773.3bA
BRS 321	1,853.1aA	8,918.0aA	11,301.1bA
BRS 322	1,620.7aB	10,408.7aA	15,537.4bA
BRS 323	1,679.7aC	15,268.0aB	46,269.9aA
BRS 324	3,058.8aB	9,935.0aB	35,159.6aA
BRS 387	4,908.8aB	11,946.0aB	54,735.6aA
Helio 250	7,055.7aA	18,074.7aA	4,450.7bA
Helio 251	5,306.7aA	11,544.0aA	5,753.7bA
Aguará 4	2,246.4aA	13,621.0aA	4,729.7bA
Aguará 6	3,897.5aA	13,186.0aA	14,617.1bA
Olisun 3	3,817.4aA	11,288.3aA	6,980.3bA
CF 101	3,338.0aA	14,063.7aA	3,259.3bA
Average	3,472.0B	12,626.4A	17,408.5A

[(1)]Means followed by equal letters, lowercase in the columns or uppercase in the rows, do not differ by the Scott-Knott test, at 5 % probability.

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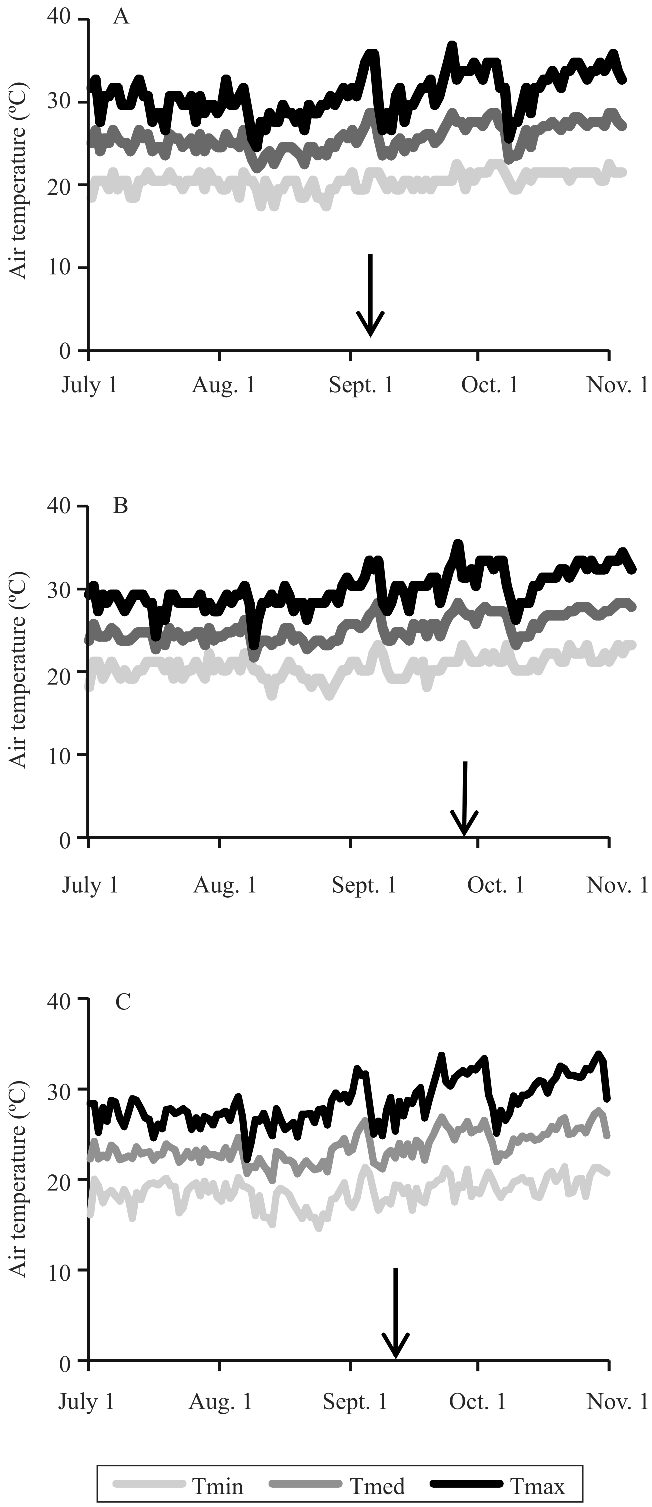

Figure 1. maximum, mean, and minimum temperatures every ten-day period, in the experimental areas, between July and October, in Poço Redondo (a), Paripiranga (b), and Frei Paulo (c). Arrows indicate when samples were removed from plants to determine proline content. Source: Agritempo (2017).

increases due to water stress (Manivannan et al., 2007; Canavar et al., 2014).

No significant difference was observed between the mean proline content of the plants grown in Poço Redondo and Paripiranga, although, in the first location, the plants had received a supplementary irrigation (Table 1). This may be explained by the volume and frequency of watering used to mitigate the water deficit in Poço Redondo, which was probably not sufficient to prevent the occurrence of drying periods before and after the irrigation events. Plants may have experienced a succession of drying periods between the irrigation events, and, consequently, a cyclic water stress of variable intensity, which may have lead to an acclimation process (Casadebaig et al., 2008). According to Conroy et al. (1988), plants are more tolerant to water deficit when water is withheld under conditions that favor the osmotic adjustment, which occurs either after previous acclimation to drought, or when water deficits are slowly imposed. In addition, the acclimation tends to lower the plant sensitivity to water stress (Casadebaig et al., 2008).

Proline contents of sunflower cultivars grown in Frei Paulo or in Poço Redondo did not differ significantly. However, proline content in cultivars grown in Paripiranga varied significantly. As water availability in Frei Paulo and Poço Redondo were higher than in Paripiranga, this result suggests that a condition of severe stress is necessary to identify which cultivars accumulate more proline. The higher levels of proline in Paripiranga were observed in BRS387, BRS323, and BRS324 cultivars (Table 1). According to Ghaffari et al. (2012) and Khalil et al. (2016), greater contents of proline contribute to a higher-osmotic adjustment, and the greatest proline accumulation in response to water deficit might be related to a competitive ability in semiarid areas (Canavar et al., 2014).

BRS387, BRS323, and BRS324 cultivars show a higher-proline content than the others evaluated in the present work, when cultivated under severe water stress in the Brazilian semiarid region.

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