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Abscisic acid induced seed dormancy and climate resilience in fox tail millet *(Setaria italica* L.) genotypes

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Abstract: A laboratory experiment was conducted at Department of Seed Science and Technology, UAS Raichur to estimate ABA content in foxtail millet (*Setaria italica* L.*)* using Phytodetek ABA Test Kit. ABA estimation in millets is helpful to trace out the reason behind the dormancy in millets and is less explored. Nine genotypes were studied in the present investigation. Among the foxtail millet genotypes, the highest dormancy duration of 35 days was observed in two genotypes *viz.,* DHFt-4-5 and DHFt-5-3 and slight dormancy was noticed in the genotype DHFt-35-1. The genotype DHFt-35-1 recorded lowest ABA concentration of 3.199 pmol/g f. w. followed by genotypes DHFt-2-5 and DHFt-2-5-1 (3.266 and 3.291 pmol/g f. w. respectively). Highest ABA concentration was found in DHFt -5-3 (3.404 pmol/g f. w.) followed by DHFt-4-5 (3.396 pmol/g f. w.). Thus it was concluded that ABA in millet seeds makes them 'climate smart crops' and during the climate change regime, it is only millets that can ensure India's food and nutrition needs in future.

Keywords: Abscisic acid (ABA), Climate change, Dormancy, Foxtail millet

INTRODUCTION

Seed dormancy has been naturally modified by changing environment and promotes germination at an undesirable time. It is reported in most of the millets. Millets possess advantages such as early maturing, drought tolerance, require minimal purchased inputs and mostly free from biotic and abiotic stresses (Annesh, 2007). Foxtail millet (S*etaria italica* L.) is one among the six small millets. It is cultivated in India, China, Eastern Europe, Southern parts of USSR and to some extent in African and American countries for hay, pasture and food grain. It is suitable for inclusion in multiple or intercropping systems because of its short duration and adjustable to mid season correction. Dormancy of foxtail millet is generally attributed to abscisic acid and its persistent lemma and palea (Jack Dekker, 2003). ABA is a plant hormone which is indispensible for development and maturation of high quality seeds. It regulates the interconnected molecular processes that control seed development, maturity, dormancy release and germination and most importantly combat the stress conditions. ABA auxotrophs of many plant species exhibit enhanced germination potential and sometimes produce viviparous seeds (McCarty, 1995), whereas mutants and transgenic lines that over accumulate ABA show enhanced dormancy (Thompson *et al.,* 2000). It has an important role in stress tolerance especially dessication tolerance (Julio Maia *et al.,* 2014). So, identification of genotypes with high ABA content will serve as a climate change mitigation strategy. With this objective, a laboratory experiment was conducted at UAS Raichur to quantify the ABA content in nine foxtail millet genotypes. The outcome of the experiment will help to identify the genotypes with high ABA content which will be suitable for cultivation in climate change regime.

MATERIALS AND METHODS

Nine foxtail millet genotypes *viz.,* DHFt-4-5, DHFt-35 -1, DHFt-55-3, DHFt-2-3, DHFt-109-3, DHFt-2-5, DHFt-2-5-1, DHFt-5-3 and Sia-326 (C) with different duration of maturity were obtained from Agricultural Research Station, Hanumanamatti, University of Agricultural Sciences, Dharwad. Experiment was conducted during the period 2013-2014 at Department of Seed Science and Technology, University of Agricultural Sciences, Raichur.

Top of paper method of germination test as prescribed by the International Seed Testing Association (1996) was followed. Four replications of 100 seeds each were randomly counted and placed on two layers of filter paper at uniform spacing in circular manner in petri dish (D=10cm). Then petri dish was covered with lid and placed in cabinet of seed germinator by maintaining a constant temperature of 25 ± 1 °C and relative humidity of 90%. The germination was recorded on seventh day and based on normal seedlings

produced; the percentage germination was worked out. Genotypes with germination percentage less than 75 per cent were considered as dormant.

ABA estimation

Sample preparation: ABA was extracted from 0.1 g of seed samples with 500 µl buffer solution (80 ml methanol, 2 ml glacial acetic acid and 18 ml double distilled water). The ground seeds were homogenized using the homogenizer and samples were centrifuged at 10,000 rpm for 10 min and then supernatant was lyophilized using the lyophilizer.

ABA-Assay methodology

Tracer Solution preparation: The tracer solution was prepared by adding 5 ml of 1X TBS buffer to each ABA tracer vials and replaced with the cap and contents were mixed by inverting the bottle several times and solution was kept for 5 min before use.

Standard preparation: For standard preparation, the standard strip was diluted with TBS per 1 ml as per the instruction in the Phytodetek ABA Test Kit. The strip was cut at the arrows so that the filter fit inside and dispensed 1 ml of 1X TBS buffer and closed the tube, vortexed the solution for an additional 30 sec. The solution approximately contained 1000 picomoles per ml (nm) ABA. The standards were serially diluted in 1X TBS buffer. The standard contained an enantiomeric mixture of (+/-) ABA. To the test wells 100 µl of standard or sample extract was added in duplicate. 100 µl of diluted tracer prepared was dispensed in each well and mixed the contents by gently tapping the plate. Test wells were covered with plate sealer and placed in a humid box (Air tight plastic box lined with damp paper towel). Test wells were incubated in the refrigerator at 4 °C for 3 hr. At the end of the incubation period, substrate was prepared by dissolving 1 substrate tablet in 5 ml of substrate diluents buffer. 100 µl of substrate was added to each test wells and incubated for 3 hr. The test wells were removed from the refrigerator and contents were expelled from the test wells. The test wells were washed twice with 1X PBST wash buffer. 200 µl of substrate solution was added to each well using a multi channel pipette, then covered the test wells with the plate sealer and placed in a humid box, and incubated at 37 °C for 60 min. The absorbance values were recorded at 405 nm by using an enzyme-linked immunosorbent assay (ELISA) reader. All determinations were carried out in dim light. The ABA levels were consistent with the dilution made and no interference from impurities was detected when ABA standards were added to diluted extracts. Results are expressed as picomol per gram fresh weight (pmol g^{-1} f. w) (Serrano *et al*., 1995).

The mean values of the data were statistically analyzed following completely randomized factorial design (CRFD) for laboratory studies; significance was tested by referring to `F' table of Fisher and Yates (1963).

Table 1. Germination percentage and ABA concentration in foxtail millet genotypes.

Genotypes	Germination (%)	ABA concentration (pmol/g f. w.)
DHFt-35-1	44.67	3.199
DHFt-2-5	44.67	3.266
DHFt-2-5-1	42.67	3.291
Sia-326 (C)	42.00	3.319
DHFt-2-3	40.67	3.334
DHFt-109-3	38.00	3.339
DHFt-55-3	36.00	3.359
DHFt-4-5	33.33	3.396
DHFt-5-3	20.67	3.404
Mean	38.07	3.323
S.Em±	0.016	0.002
CD at 1%	0.049	0.004

RESULTS AND DISCUSSION

Germination percentage: All the nine genotypes were found to possess dormancy as none of the genotypes recorded the required minimum germination standard of 75 per cent. The highest germination percentage was recorded in DHFt-35-1 and DHFt-2-5 (44.67 %). The lowest germination percentage of 20.67 per cent was recorded in DHFt-5-3 (20.67 %) followed by DHFt-4-5 (33.33 %) ((Table 1).

ABA content: ABA concentration in the foxtail millet genotypes varied from 3.195 to 3.404 picomoles per gram fresh weight (pmol/g f. w.) of seed (Table 1). The genotype DHFt-35-1 recorded lowest ABA concentration of 3.199 pmol/g f. w. followed by genotypes DHFt-2-5 and DHFt-2-5-1 (3.266 and 3.291 pmol/g f. w. respectively). Highest ABA concentration was found in DHFt-5-3 (3.404 pmol/g f. w.) followed by DHFt-4-5 (3.396 pmol/g f. w.).

In plants, the phytohormone abscisic acid (ABA) plays a major role in the responses to a wide range of stresses, including drought, high salinity, and low temperature, and in developmental processes such as seed maturation, dormancy, and germination. Loss and re-establishment of dessication tolerance in germinated Arabidopsis seeds was studied in ABA-deficient and ABA-insensitive mutants by Julio Maia *et al*. (2014) and demonstrated that the developmental window in which dessication tolerance can be re-established correlates strongly with the window in which ABA sensitivity is still present. Cellular dehydration induced by seed maturation and drought stress increases plant ABA levels. ABA induces dormancy in seeds by blocking germination and promoting the synthesis of storage proteins. Plants adapted to temperate climates require a long period of cold temperature before seeds germinate. This mechanism protects young plants from sprouting too early during unseasonably warm weather in winter. As the hormone gradually breaks down over winter, the seed is released from dormancy and germinates when conditions are favorable in spring. Another effect of ABA is to promote the development of winter buds; it mediates the conversion of the apical meristem into a dormant

bud. Exogenous application of ABA was able to increase plant adaptive response to various environmental conditions. Addition of ABA (5 mg/l) to the callus of resurrection plant induced tolerance to desiccation (Bartels *et al.,* 1990).

ABA was more effective than BA and CCC to ameliorate the toxic effects of salt stress in rice (Gurmani *et al*., 2011). McWha (1975) and King (1976) found the level of ABA at its peak to be about 6 to 8 ng per grain in wheat which prevented precocious germination thereby avoiding the unfavourable climatic conditions. The level of seed dormancy in tomato at maturity is perhaps the biggest factor determining the sprouting (Thompson *et al.,* 2000). Many growth inhibiting substances like Maleic hydrazide and Abscicic acid have been found to prevent precocious germination. Three ABC-insensitive mutants of *Arabidopsis*; *abi1*, *abi2* and *abi3* have been found by Rock and Zeevaart (1991). The product of the *abi3* gene acts primarily in seed development. ABA-deficient mutants were affected in the regulation of many genes by drought, salt, and cold. Crop duration and fresh seed dormancy in Spanish varieties of ground nut are important adaptation traits. *In situ* germination, a consequence of lack of fresh seed dormancy leads to pod yield and quality loss in rainfed environments, particularly when rains coincide with the crop maturity stage (Pasupuleti Jenila *et al.,* 2013). Yana Kazachkova *et al*. (2015) observed that levels of ABA were significantly higher while levels of GA were lower in salt-treated *Thellungiella salsuginea* seeds. They concluded that this germination system may serve as a seed survival strategy under unfavourable conditions. Spray and drench applications s-ABA to impatiens, seed geranium, petunia, marigold, salvia, and pansy was consistently effective at reducing water loss and extending shelf life for all species treated (Nicole Waterland *et al.*, 2010).

Conclusion

It can be concluded that dormancy in foxtail millet is due to ABA. Genotypes with high ABA content was identified and it will be suitable for cultivation in climate change regime. The disadvantages of seed dormancy weigh more than the advantages. But, our study concludes that ABA induced seed dormancy is the need of the hour as a climate mitigation strategy. Identification of genotypes with high ABA content will help to incorporate dormancy trait to crop plants through breeding programmes. Abscisic acid content will serve as an indicator for the selection of the suitable genotypes for cultivation in adverse climatic conditions, which is not so explored especially in minor millets.

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