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International Journal of Current Research
Vol. 7, Issue, 08, pp.19386-19390, August, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ABA PRODUCTION IN RESPONSE TO THE DESICCATION OF RECALCITRANT EMBRYOS OF *ARTOCARPUS HETEROPHYLLUS* LAM. AND *ARTOCARPUS HIRSUTUS* LAM

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ARTICLE INFO

Article History:
Received 15th May, 2015
Received in revised form
27th June, 2015
Accepted 10th July, 2015
Published online 31st August, 2015

Key words:
ABA, *Artocarpus heterophyllus*, *Artocarpus hirsutus*, Indirect competitive ELISA, Orthodox seeds, Recalcitrant seeds, Desiccation-sensitive, Desiccation-tolerant.

ABSTRACT

The phytohormone, ABA plays a vital role in the growth and development of plants. The hormone is generally associated with seed dormancy and it is reported to be involved in desiccation tolerance in orthodox seeds. There is no conclusive information about the role of ABA in recalcitrant species. The present investigation was aimed at understanding the changes in ABA level during the desiccation of recalcitrant embryos of *Artocarpus heterophyllus* Lam. and *Artocarpus hirsutus* Lam. A significant positive correlation has been established between moisture content and ABA level in the local cultivars of *Artocarpus heterophyllus* (viz., *varikka* and *koozha*). A comparatively high level of ABA could be observed in the mature embryos of both cultivars of *Artocarpus heterophyllus*. Vivipary has been observed in these cultivars even under elevated levels of ABA. The ABA level drastically decreased during desiccation of embryos. However, the embryos could tolerate mild desiccation even under low level of ABA. A different trend in ABA could be observed in *Artocarpus hirsutus* in which the embryos maintained extremely low level of ABA and could tolerate desiccation better than *Artocarpus heterophyllus*. No significant correlation has been established between moisture content and ABA level in this species. Vivipary could not be observed in *Artocarpus hirsute s*even under low levels of ABA. Results suggest that vivipary cannot be attributed to the low level of ABA and it is not the sole factor in providing desiccation tolerance in orthodox seeds.

Citation: Ajith Kumar, K. G., Sunil Kesavadeth, G., Pratheepkumar, V., Bindumole, V. R., Aswathy, P. J., Athira, V. R. and Anju, T. U., 2015. "ABA production in response to the desiccation of recalcitrant embryos of *Artocarpus heterophyllus* LAM. and *Artocarpus hirsutus* LAM", *International Journal of Current Research*, 7, (8), 19386-19390.

INTRODUCTION

Abscisic acid (ABA) is a versatile plant growth regulator known to influence many physiological processes in plants. It is the major growth inhibitor in plants responsible for seed and bud dormancy. This hormone plays a key role in preparing embryos for maturation drying before dormancy. There are several reports indicating the association between ABA levels and desiccation tolerance in orthodox species (Quatrano, 1987). Farnsworth (2000) reported that in desiccation-tolerant orthodox seeds, the ABA peaks either once or twice during embryogenesis and these peaks coincide with the onset of maturation drying which is a pre-requisite to dormancy and subsequent germination. However, recalcitrant species are desiccation-sensitive that are unable to tolerate dehydration of embryos below a threshold level. Many of the tropical tree species are recalcitrant and it has been a challenge to the survival of these species. The exact reason behind the evolution of recalcitrance in plants is largely unknown. Most of the studies in the embryogeny of orthodox seeds showed a characteristic trend in the production of ABA, tolerance to desiccation and the subsequent germination. However, such a trend could not be established in recalcitrant species.

Only a few reports are available indicating the sensitivity of recalcitrant embryos to desiccation. Some evidences suggest that in recalcitrant species such as *Theobroma cacao* (Pence., 1991), *Quercus rober* (Finch-Savage, 1992), *Hopea odorata* (Garcia *et al*., 1998) and *Machilus thunbergii* (Lin *et al*., 1995) low level of ABA is maintained throughout embryo development and it might be the reason for desiccation sensitivity in these species indicating the importance of ABA in developing desiccation tolerance in species. Trewavas (1987) highlighted that more information on hormonal sensitivity is needed for a wide range of recalcitrant species to understand why there is a lack of desiccation tolerance in recalcitrant species.

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Two recalcitrant tree species; *Artocarpus heterophyllus* Lam. (cultivars; *varikka* and *koozha*) and *Artocarpus hirsutus* Lam. were selected for the study. The major objective of the present investigation was to understand the change in ABA level during the desiccation of these recalcitrant embryos and to draw a characteristic trend, if any, in ABA production.

MATERIALS AND METHODS

The mature fruits of *Artocarpus heterophyllus* Lam. (local cultivars; *varikka* and *koozha*) and *Artocarpus hirsutus*Lam. were collected during the month of June, 2015. The fruits were de-pulped and ten fresh seeds were randomly taken from the seed lot to determine the moisture content by oven dry method. Fifty randomly selected seeds were placed in the growth chamber to understand the viability of fresh and mature seeds. Five fresh seeds were randomly selected for ABA immunoassay. Remaining seeds were allowed to dehydrate under natural conditions at different intervals of time and the samples were collected as described earlier.

ABA Extraction

The extraction method was a modification of Weiler (1982) as described by Wolf, Jeschke and Hartung (1990). The embryos of five selected seeds were chopped together and 50mg of embryonic tissue was taken with a replication of three. The tissue was ground with a mortar and pestle in cold 90% methanol and a few drops of BHT was added to it and the samples were kept at 4^0C for 48hours. The samples were centrifuged at 3000g for 10min and the supernatant was collected. It was then passed through C_{18}Sep-pac column to remove the pigments and other lipophilic impurities. The methanol in the sample was removed by vacuum evaporation and the residue was diluted in 1ml distilled water.

ABA Immunoassay

The Indirect competitive ELISA developed for ABA assay by Folkard Ash (2000) was followed in this study. The ABA-BSA conjugate, IgG primary antibodies raised in rabbit against the conjugate and the alkaline phosphatase labeled secondary antibody raised in goat against the primary antibodies were procured from Agrisera, Sweden and all other chemicals were bought from Sigma, USA. Each well received 0.2mL of a 20µg mL^{-1}IgG solution in 50Mm $NaHCO_3$ with a pH 9.6. The enzyme conjugate dilution was chosen so that, under the assay conditions described, absorbance **A** 405 = 1.0 for 100% binding, was obtained after 1h at 37^0C. Under these conditions, non-specific binding was less than 5% (equivalent to A 405 = 0.05). The OD values were plotted against their ABA standards and a linear regression equation was developed to determine the quantity of ABA in the sample.

Statistical Analysis

The data were subjected to analysis of variance, correlation analysis and regression analysis using the Statistical Package for Social Sciences version 12.0 (Windows, SPSS Inc).

RESULTS AND DISCUSSION

Significant changes in the endogenous ABA level have been observed in the embryonic tissues of both recalcitrant species during desiccation. In *Artocarpus heterophyllus* (cult.*varikka*), the ABA content was 4.681nmol $gfwt^{-1}$ at maturity of the embryo (Table 1/Fig 1). The moisture content of the embryonic tissue was 71.27% and cent percent germination of seeds could be achieved in this moisture content. When the moisture content decreased to 60.73% during desiccation, the ABA content also dropped to 3.365nmol $gfwt^{-1}$. The same trend continued when the moisture content of embryonic tissue decreased to 55.73% and the ABA dropped to 0.648nmol $gfwt^{-1}$. Cent percent germination of seeds could be observed during this initial phases of desiccation indicating the insensitivity of seeds. A further decrease in moisture content of embryonic tissue was found to influence the germination of seeds of this cultivar. 80% seed germination could be obtained when the moisture content decreased to 51.45% and at the same time the endogenous ABA level was as low as 0.380nmol $gfwt^{-1}$. A positive correlation (r = 0.949*) could be established between the moisture content and the ABA level in the embryonic tissue of this cultivar.

Artocarpus heterophyllus (cult.*koozha*) also exhibited the same trend as shown by the cultivar *varikka*. The ABA content in the embryonic tissue was found to be decreasing with decrease in moisture content (Table 1, Fig 2). The ABA content in the mature embryo of the cultivar *koozha* was as high as 316.05nmol $gfwt^{-1}$ at a moisture content of 62.6%. Cent percentage seed germination could be obtained when the moisture content decreased to 56.9% indicating the insensitivity of this cultivar towards slight desiccation.

Table 1. Change in ABA content and the percentage of germination in response to desiccation in recalcitrant species

Recalcitrant species	Satge of seed	Moisture content (%)	ABA (nmol g fwt^{-1})	Germination (%)
	Mature	71.27±0.80	4.681±0.41*	100
Artocarpus heterophyllus	Desiccated (3 days)	60.73±0.68	3.365±0.28*	100
(Cult. *Varikka*)	Desiccated (6 days)	55.73±0.65	0.648±0.012*	100
	Desiccated (9 days)	51.45±0.61	0.38±0.011*	80
	Mature	62.6±0.71	316.05±23.65*	100
	Desiccated (4 days)	59.38±0.66	163.44±12.98*	100
Artocarpus heterophyllus	Desiccated (8 days)	56.9±0.63	51.43±6.21*	100
(Cult. *Koozha*)	Desiccated (10 days)	54.65±0.61	0.375±0.15*	60
	Desiccated (14 days)	51.12±0.60	12.77±0.98*	20
	Mature	47.21±0.49	0.452±0.52*	100
Artocarpus hirsutus	Desiccated (3 days)	36.5±0.41	0.027±0.002*	100
	Desiccated (6 days)	31.49±0.39	0.07±0.003*	100
	Desiccated (9 days)	26.68±0.38	0.903±0.005*	20

* values are significant at 5% level.

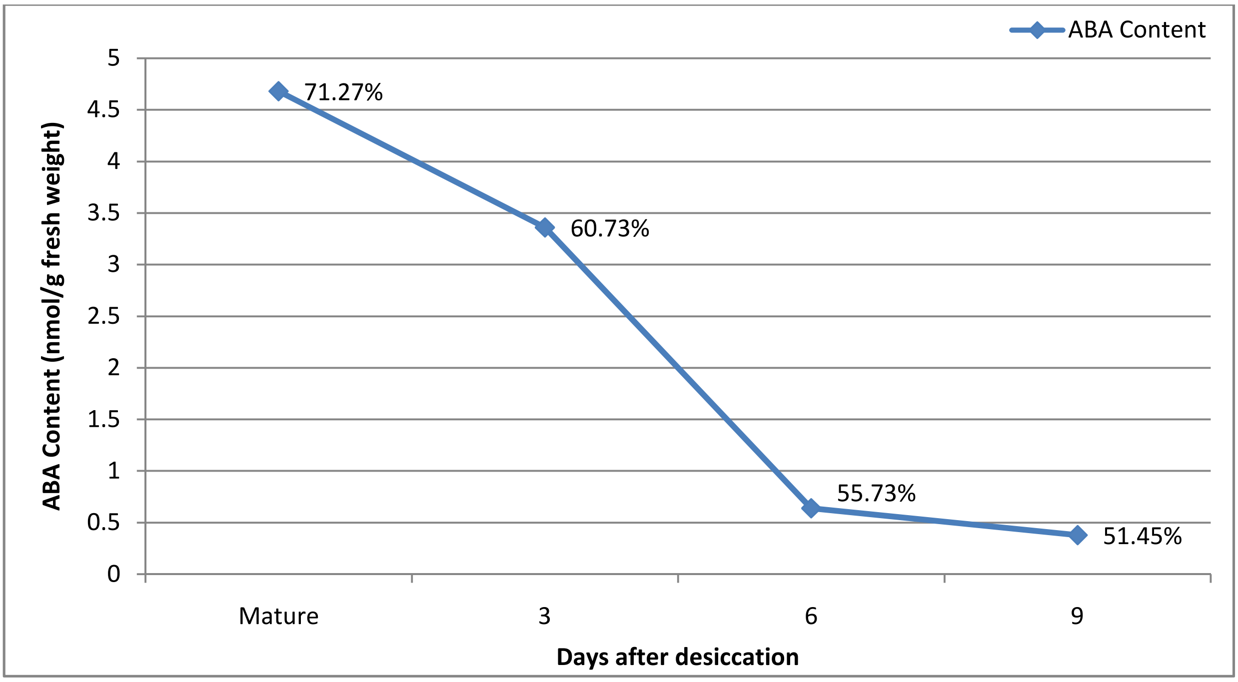

Figure 1. Change in ABA level and moisture content during the desiccation of the seeds of *Artocarpus heterophyllus* (cult. *varikka*)

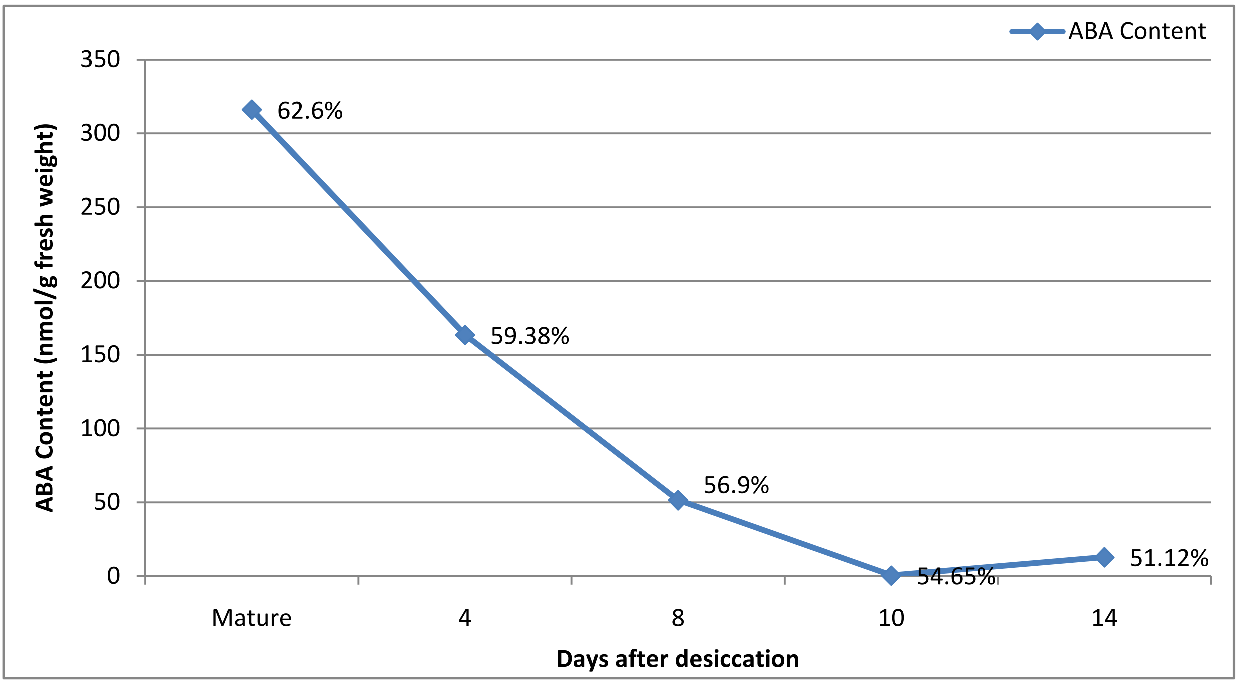

Figure 2. Change in ABA level and moisture content during the desiccation of the seeds of *Artocarpus heterophyllus* (cult. *koozha*)

However, further decrease in moisture content negatively influenced seed germination. Only 20% seed germination could be observed when the moisture content decreased to 51.12%. This indicates that the threshold water level (minimum water required for cent percent germination) is ~ 55% for both cultivars of *Artocarpus heterophyllus*. At the same time, a significant amount of ABA (12.77nmol gfwt^{-1}) could be seen even in this dehydrated embryonic tissue of the cultivar, *koozha*. A similar positive correlation (r= 0.909*) could also be established between the moisture content and the ABA content in the embryonic tissue of this cultivar.

A comparatively low level of ABA could be observed in the mature and desiccated seeds of *Artocarpus hirsutus* (Table 1/Fig 3). The mature seeds showed 0.452nmol gfwt^{-1} of ABA in the embryonic tissue at a moisture content of 47.21% and cent percent germination could be obtained. The ABA level was found to decrease from 0.452nmol gfwt^{-1} to 0.027nmol gfwt^{-1} when the moisture content in the embryo dropped to 36.50% without any change in the seed viability (cent percent germination). However, a significantly higher level of ABA could be observed in highly desiccated seeds (moisture content of 26.68%) but the seed viability was greatly impaired (only

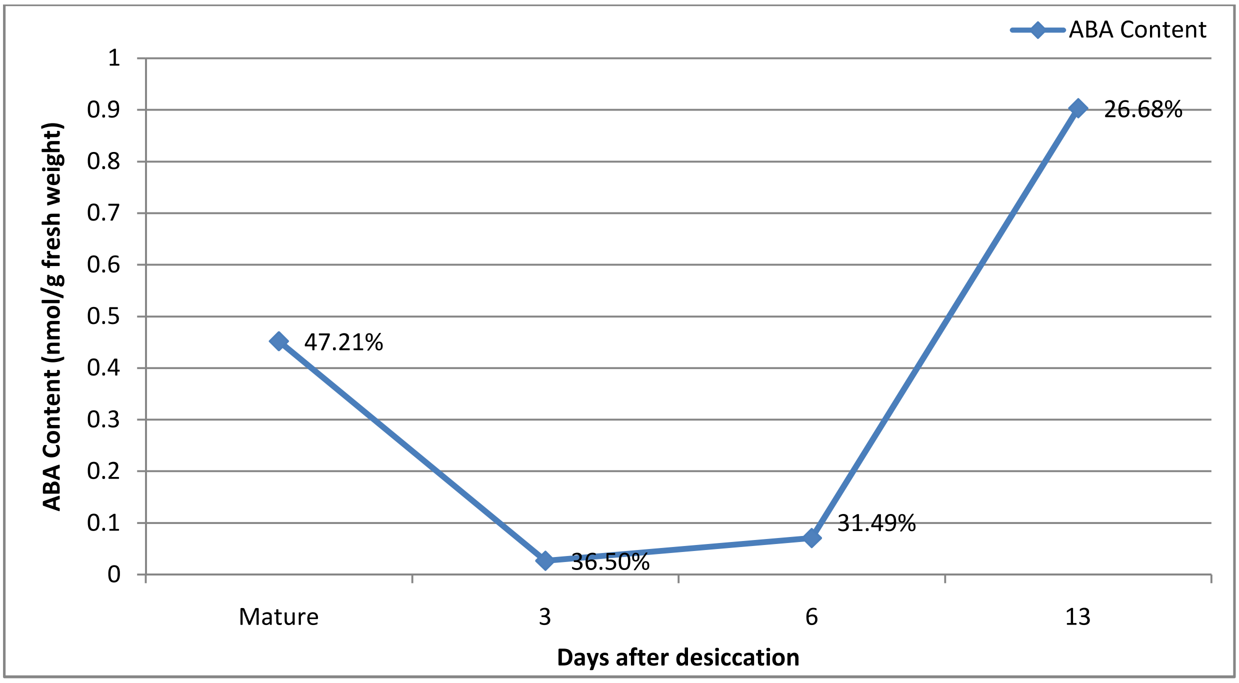

Figure 3. Change in ABA level and moisture content during the desiccation of the seeds of *Artocarpus hirsutus*

20% germination could be obtained). The threshold water content was ~31% for cent percent germination indicating the ability of the embryos of this species to tolerate desiccation better than *Artocarpus heterophyllus*. However, an insignificant correlation (r= - 0.268) has been established between moisture content and ABA level in this species. The results of the present study indicate that embryonic tissues of both recalcitrant species could produce ABA even under desiccation but a general trend could not be deducted from the results. However, a decreasing trend in ABA production during desiccation could be observed in both cultivars of *Artocarpus heterophyllus*. This is contrary to the reports on other recalcitrant species such as *Theobroma cacao* (Pence VC., 1991), *Quercus rober* (Finch-Savage, 1992), *Hopea odorata* (Garcia *et al*., 1998) and *Machilus thunbergii* (Lin *et al*., 1995) in which a low level of ABA is maintained throughout embryogenesis. The species *Artocarpus heterophyllus*is characterized by viviparous germination in which the seeds germinate at maturity within the fruit.

Generally, viviparous germination is linked to lower level of ABA in the embryonic tissue (Neill *et al*., 1986; Fong *et al*., 1983). This raises a question about the vivipary in *Artocarpus heterophyllus* even under elevated levels of ABA in the embryonic tissues of mature seeds. The ABA content in the mature embryonic tissue was comparatively low in *Artocarpus hirsutus* but vivipary could not be observed in this species. No precise explanation can be given to this problem but it can be argued that ABA might not have any link with vivipary. A drop in ABA level in the embryonic tissue could be observed during the desiccation of the embryos of both cultivars of *Artocarpus heterophyllus* and a comparatively low level of ABA in the embryos of *Artocarpus hirsutus*. There are several reports indicating the role of ABA in the development of desiccation tolerance in orthodox seeds (Koornneef *et al*., 1989; Neill *et al*., 1986; Fong *et al*., 1983).

The loss of cell turgor or cell membrane perturbation accompanied by dehydration is considered as a signal for ABA accumulation and subsequent desiccation tolerance (Ackerson RC., 1983; Milborrow BV., 1973; Quarrie SA., 1984; Zeevaart JAD., 1980). The studied species showed tolerance to mild desiccation. Both cultivars of *Artocarpus heterophyllus* could tolerate desiccation up to 55% even under low level of ABA. However, *Artocarpus hirsutus* could tolerate desiccation up to 31% even under extremely low level of ABA. This indicates that ABA is not the sole factor responsible for providing tolerance to desiccation. Further research is required to understand the molecular mechanism behind the sensitivity of recalcitrant seeds towards desiccation.

Conclusions

The following conclusions are drawn from the study

- The two local cultivars of *Artocarpus heterophyllus* viz., *varikka* and *koozha* show vivipary even under elevated levels of ABA.
- The species *Artocarpus hirsutus* does not show vivipary even under lower levels of ABA
- The ABA level is found to decrease during the desiccation of the embryos of both cultivars of *Artocarpus heterophyllus*. A significant positive correlation exists between the moisture content and ABA content in these cultivars. However, the correlation is insignificant in *Artocarpus hirsutus.*
- Cent percent germination is obtained in the mature, just fallen seeds of *Artocarpus heterophyllus* (both cultivars) and *Artocarpus hirsutus*. However, the percentage of germination decreases with the desiccation of embryos.
- The threshold water level (minimum water required for cent percent germination) is approximately 55% for both cultivars of *Artocarpus heterophyllus* and 30% for *Artocarpus hirsutus*.

Acknowledgement

The authors greatly acknowledge the financial assistance provided the University Grants Commission, New Delhi, India for the successful completion of this study.

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