BIOLOGIA PLANTARUM 52 (1): 181-183, 2008

BRIEF COMMUNICATION

Effect of abscisic acid and proline on *in vitro* flowering in *Vigna aconitifolia*

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Abstract

An experiment was taken up to find out possibilities of manipulating the *in vitro* flowering in moth bean. Abscisic acid (ABA) and proline both alone and in combination influenced days to flower induction, number of flowers per plant, number of pods per plant and seeds per pod. Frequency of flowering plants approached 100 % at 1 and 3 μM ABA and 800 μM proline. The range of flowering period (3 to 23.6 d) has also been influenced by various treatments.

Additional key words: moth bean, flowering induction, water stress, stress hormone, precocious flowering, callus, pods, plant height.

Regulation of flowering in *in vitro* cultures is important practically as well as theoretically for understanding of mechanism. Growth regulators along with environmental factors and physiological state of the plant have been indicated to influence *in vitro* flower induction (Narsimhulu and Reddy 1984, Rastogi and Sawhney 1989, Nadgauda *et al.* 1990). There are many suggestions in the literature that water and heat stress might interact directly with the reproductive process adjusting sensitive reproductive stages to periods of reduced stress (Chiariello and Gulman 1991, Southwick and Davenport 1986). At the same time, different environmental stresses lead to elevated ABA concentration. ABA is considered to be a stress hormone and functions through a set of ABA regulated genes, which in turn lead to accumulation of osmo-protectants like proline. In addition, there were indirect evidences that stress induced compounds like abscisic acid (ABA, Tanimoto *et al.* 1985) and proline (Deotale *et al.* 1988, Virupakshi *et al.* 2002) may influence flowering. This was taken as a hypothesis for present investigation that ABA or proline may induce flowering in *in vitro* cultures of moth bean (*Vigna aconitifolia*), a highly drought tolerant crop species.

Seeds collected from single plant of *Vigna aconitifolia* J. Marechal cv. IPCMO 880 were surface sterilized with 0.1 % mercuric chloride for 4 min and thoroughly washed with sterilized distilled water for 4 - 5 times. These surface sterilized seeds were germinated in test tubes on a filter paper dipped in distilled water. Apical shoot of 7 to 8 d old seedlings measuring 2.0 - 2.5 cm comprising two fully expanded primary leaves and young primordial leaves along with shoot tip were cut and inoculated in glass jam bottles. The Murashige and Skoog (1962; MS) medium supplemented with various concentrations of ABA (0.1, 1.0, 3.0 and 5.0 μM) and proline (200, 400, 600 and 800 μM) individually and in combination along with sucrose 30 g dm^{-3} and agar 8 g dm^{-3} was used for culture. The glass bottles were incubated in a culture room at temperature of 27 ± 0.5 °C and 14-h photoperiod. Uniform irradiance of 68 μmol m^{-2} s^{-1} was obtained from fluorescent tubes (*Philips* 40 Watt). Observations were recorded on ten plants for plant height, number of branches, number of days to flower induction from date of inoculation, number of flowering plants, number of flowers per plant, number of pods per plant and germination ability of *in vitro* obtained seeds.

The addition of ABA and proline either alone or in combination enhanced flowering by approximately 15 to 20 d in moth bean. However, all of the treatments were not equally effective in their enhancing effect on flowering (Table 1). Independent treatments either with ABA or proline were more effective in advancing the flowering and increased the number of flowering plants, up to 100 % for 1 and 3 μM ABA and 800 μM proline. Similarly, the number of flowers per plant was also increased by ABA and proline over control (2.5). In general 2 - 5 times increase in number of flowers was observed for various treatments. The increase in flower number, however, did not result in higher number of pods

Received 10 January 2007, *accepted* 20 December 2007.
Abbreviations: ABA - abscisic acid; MS - Murashige and Skoog.
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Table 1. Effect of ABA and proline on different morphological traits under *in vitro* conditions. Means ± SE, $n = 10$.

	Conc. [μM]	Time to flowering [d]	Number of flowers	Number of flowering plants	Flowering period [d]	Number of pods [plant]	Number of grains [pod^{-1}]	Plant height [cm]	Number of branches
Control		70.0±0.88	2.5±0.10	2.0±0.33	10.0±0.56	2.5±0.08	2.5±0.04	11.9±0.19	3.7±0.15
ABA	0.1	53.1±0.88	6.5±0.10	7.0±0.33	14.1	3.6±0.08	3.2±0.04	3.8±0.19	3.8±0.15
	1.0	53.3±0.88	7.9±0.10	10.0±0.33	14.0	2.6±0.08	2.6±0.04	3.6±0.19	4.0±0.15
	3.0	55.4±0.88	7.1±0.10	10.0±0.33	18.7	2.6±0.08	2.6±0.04	2.9±0.19	3.0±0.15
	5.0	64.7±0.88	5.0±0.10	4.0±0.33	7.0	3.5±0.08	2.3±0.04	2.3±0.19	2.4±0.15
Proline	200	60.6±0.88	9.5±0.10	4.0±0.33	15.5	1.6±0.08	2.7±0.04	10.8±0.19	4.8±0.15
	400	60.5±0.88	6.8±0.10	6.0±0.33	12.1	2.0±0.08	2.3±0.04	5.4±0.19	4.2±0.15
	600	50.6±0.88	7.6±0.10	6.0±0.33	18.8	2.0±0.08	3.2±0.04	5.3±0.19	4.6±0.15
	800	48.7±0.88	5.9±0.10	10.0±0.33	19.5	2.4±0.08	2.5±0.04	5.7±0.19	5.5±0.15
ABA	0.1+200	51.0±1.97	9.5±0.22	2.0±0.75	18.5±1.25	0.0±0.19	0.0±0.10	10.7±0.44	6.4±0.35
+proline	0.1+400	00.0±1.97	0.0±0.22	0.0±0.75	0.0±1.25	0.0±0.19	0.0±0.10	11.9±0.44	6.2±0.35
	0.1+800	56.0±1.97	5.0±0.22	1.0±0.75	14.0±1.25	0.0±0.19	0.0±0.10	11.4±0.44	6.1±0.35
	1.0+200	57.0±1.97	4.0±0.22	1.0±0.75	9.0±1.25	0.0±0.19	0.0±0.10	10.1±0.44	4.9±0.35
	1.0+400	54.8±1.97	7.4±0.22	8.0±0.75	15.2±1.25	2.0±0.19	2.7±0.10	9.6±0.44	4.9±0.35
	1.0+800	45.5±1.97	12.0±0.22	5.0±0.75	23.6±1.25	1.5±0.19	3.0±0.10	9.8±0.44	5.5±0.35
	3.0+200	52.3±1.97	13.5±0.22	2.0±0.75	22.5±1.25	0.0±0.19	0.0±0.10	8.5±0.44	3.7±0.35
	3.0+400	54.5±1.97	7.0±0.22	2.0±0.75	15.0±1.25	0.0±0.19	0.0±0.10	8.1±0.44	3.2±0.35
	3.0+800	69.5±1.97	2.0±0.22	2.0±0.75	3.0±1.25	0.0±0.19	0.0±0.10	10.0±0.44	3.5±0.35
CD 5 %		5.78	0.66	2.18	3.69	0.58	0.32	1.21	0.96

or seeds per pod. Most of plantlets treated by combination of ABA and proline did not bear pods and seeds while bearing considerably more flowers than controls. The seeds developed *in vitro* looked normal and germinated on filter paper dipped in MS liquid medium or in solidified MS medium. However, germination was lower on filter paper grown seeds (only 20 ± 6 %) compared to those on solidified MS medium (45 ± 5 %).

The promoting effect of ABA and proline on induction of *in vitro* flowering could be related with their role in water stress management. ABA (often referred to as generalized stress hormone) and osmoprotectant proline have been found to accumulate in floral parts (Deotale *et al.* 1988). Further, Leport and Larher (1988) suggested that this proline accumulation in flowers could not be solely the result of a metabolic response to water stress. Though, application of ABA might lead to accumulation of proline, this does not seem to be mechanism for enhanced flowering in the present study as rather antagonistic effects were reported in combinations of the two compounds. However, presence of ABA responsive elements (ABRE) in the genes regulated by dehydration indicated a putative role of ABA in flower induction. The transcription factors like DREB1a, DREB1b, and DREB1c, are all members of the apetala-2/ethylene response element binding protein family, originally found to control flower development and ethylene responses in *Arabidopsis* (Riechmann and Meyerowitz 1998). In addition to our observations, there are few reports available indicating the role of ABA in *in vitro* (Tanimoto *et al.* 1985) or *ex vitro* (Saini *et al.* 1983) flowering.

Proline and ABA supplemented in the medium individually resulted into reduction in plant height compared to control. The reducing effect was more pronounced for ABA followed by higher doses of proline (> 200 μM). However, effect of ABA was alleviated proline addition. Yellowing of upper leaves was observed with increasing concentrations of ABA in the medium. Low concentration of ABA (0.1 μM) when combined with proline was found to promote plant height to control level, which ranged from 10.7 to 11.9 cm at all the concentration of proline (Table 1).

Application of proline alone or in combination with 0.1 - 1 μM ABA increased the number of branches (from 3.7 in control upto 6.4 at 0.1 μM ABA + 200 μM proline). Conversely, higher ABA concentrations (3 and 5 μM) reduced branching (Table 1).

This reducing effect of ABA on plant height and number of branches has been well documented (Salisbury and Ross 1992) and might be part of the stress management. The inhibitory effect of ABA on plant height and branching was reduced with the application of various concentrations of proline. Though both proline and ABA are induced during water stress, in combination their antagonistic effect on various traits, *e.g.*, flowering, plant height and branching was astonishing.

This study may help in elucidating the physiology of flowering and if applied under *in vitro* conditions, may skip tedious hardening process to rescue a desired somaclonal variant.

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