

Effect of Seed Vigor and Hormone Priming on Glyoxylate Cycle Enzymes Activity in Persian Silk Tree (*Albizia julibrissin* Durazz.)

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Abstract: In order to investigate the effects of seed vigor on glyoxylate cycle enzymes activities, seed germinability and invigorative effect of hormone priming on different vigor seed lots of 'Persian silk tree' experiments were conducted. Results indicated that the loss of seed vigor can cause the reduction of germination percentage and glyoxylate cycle enzymes activities. Priming of seeds by both two phytohormones improved and invigorated the poor vigor seed lots. The hormone-priming increased the activities of glyoxylate cycle enzymes.

Key words: Enzymes • Glyoxylate cycle • Hormone priming • Seed reserve utilization • Seed vigor

INTRODUCTION

Viable seed is capable of producing new plant under both favorable and unfavorable climatic conditions. Emergence of the seed lot having high vigor is more uniform and due to that it forms more vigorous seedlings which in turn provide better stand establishment [1]. Seed and seedling vigor a priori involves the coordinated regulation of many genes in various biochemical pathways, including mobilization of seed storage reserves. Efficient use of energy reserves to enable continuous heterotrophic growth is fundamental for rapid establishment [2]. In the germinating seed triacylglycerides are important sources of energy. On the other hand, the occurrence of a complete glyoxylate cycle in the 'persian silk tree' (*Albizia julibrissin* Durazz.) seeds is in accordance with the presence of many lipid reserves as an initial energy source during germination and seedling establishment. Various researchers confirm the important role of the glyoxylate cycle in seed germination and vigor [3-5]. Differential activity of the glyoxylate cycle appears to be a physiological marker that distinguishes between high- and low-vigor sugar beet cultivars [3]. This finding suggests, for the first time, a biochemical target for selection for enhanced germination and improved emergence in sugar beet. Seed deterioration

leads to reductions in seed vigor and seedling emergence [6, 7]. Perhaps the most frequently cited cause of seed deterioration is lipid peroxidation. When seeds are damaged by improper storage conditions, lipid degradation reactions can occur [6]. Therefore, it seems that the glyoxylate cycle enzymes activity is influenced by seed deterioration, directly and indirectly.

Repair occurs during imbibition has been used for many plants through seed priming. This process entails the hydration of seeds using various protocols followed by re-drying to permit routine handling. The benefits include increased germination rate, more uniform emergence and germination under a broader range of environments and improved seedling vigor and growth. Priming also reduces lipid peroxidation during subsequent seed storage. Addition of plant growth regulators to deteriorated seeds improves their performance [6]. The gibberellins (GA3 and GA4/7) have been beneficial in stimulating germination of several species under unfavorable conditions [8]. Singh and Amritphale [9] used organic solvents to permeate dry GA3 and benzyladenine into soybean seeds causing increased vigor of artificially and naturally aged seeds. Jeng and Sung [10] found that glyoxylate cycle enzymes (isocitrate lyase and malate synthase) were increased by priming. There are some conflicting reports on the effect of

salicylic acid (SA) on seed germination and seedling establishment, suggesting that this molecule can either inhibit germination or enhance seed vigor [11, 12]. In another study, Basra *et al.* [13] found that seed priming with SA improve the germination and early seedling growth in coarse and fine rice. In the present report, we investigated the effects of seed deterioration and enhancement on 'persian silk tree' seed germinability, vigor and glyoxylate cycle enzymes activity.

MATERIALS AND METHODS

Seed of 'persian silk tree' (*Albizia julibrissin* Durazz.) was divided to five sub-samples and a sub-sample was kept as control or high vigor seed lot (SV₁). Factorial experiment was conducted in completely randomized design (CRD) with four replications in laboratory. However, four other sub-samples with about 15-16 per cent moisture content were artificially deteriorated at 40° for the period of 10, 20, 30 and 40 days. So, the five seed lots were obtained with 83.5% (SV₁), 72.3% (SV₂), 58.1% (SV₃), 40.4% (SV₄) and 23.5% (SV₅) seed viability. Prior to the experiment, the probable seed lot dormancy was broken by pre-chilling (one month in 3°) and scarification. Seed of different vigor was primed with gibberellic acid (GA3, 20 mg L⁻¹) and salicylic acid (SA, 50 mg L⁻¹) for 24 h and also unprimed control seed lots were regarded. Seed was surface sterilized in 5 per cent sodium hypochlorite (Whitex®) solution for 15 minutes, subsequently washed with distilled water and air-dried before being used in the germination experiments. Germination was tested at 20° without photoperiod. Four replicates of 50 seed each were germinated in two folds of whatman filter paper #1 placed in 10×20cm plastic plates. A seed was considered as germinated when the radicle emerged. Germination was noted on consecutive days for 20 d. Germination rate (GR) and mean germination time (MGT) were calculated according to Ellis and Roberts [14].

Seed reserve mobilization dynamics was evaluated using the procedure of Soltani *et al.* [15]. The initial seed dry weight was calculated using the data for seed fresh weight and moisture content. After eight days of germination, oven-dried weight of 15 seedlings was determined. The weight of utilized (mobilized) seed reserve (USR) was calculated by subtracting the dry weight of the seed remnant from the dry weight of the

original seed. Seed reserve utilization efficiency (SRUE) into plant tissue was estimated by dividing seedling dry weight (SLDW) by the USR. The ratio of utilized seed reserve to initial seed dry weight was considered as seed reserve depletion percentage (SRDP). For glyoxylate cycle enzyme activity determinations, the randomly sampled seedlings were stored in liquid nitrogen. The seedlings were hand homogenized at 4° in a pre-chilled mortar and pestle (suitable sample to extraction buffer ratio) with 0.15 M Tris-HCl (pH 7.5) containing 1 mM EDTA, 2 mM DTT, 10 mM KCl, 10 mM MgCl₂, 0.6 M sucrose. The homogenate was centrifuged at 12000×g for 20 min and the supernatant served as the enzyme preparation for isocitrate lyase (ICL, EC 4.1.3.1) and malate synthase (MS, EC 4.1.3.2) activity assays [16].

Data were analyzed as a factorial experiment on the basis of completely randomized design (CRD) with 4 replications. All data subjected to an analysis of variance and when a significant (P< 0.05) F ratio occurred for treatment effects, comparison of means was carried out using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Results showed high vigor (non-deteriorated) seed lot, when was hormone-primed had the greatest germinability. The two hormones which were applied in the experiment had the same effects (Table 1). The germination percentage was decreased with reducing seed vigor level. When all seed lots of different vigor level were hormone-primed, their germination capabilities were enhanced. Parameters of GR, SRUR, SRUE and FUSR were also changed in the same procedure as germination (Table 1).

The hormone-priming increased the activities of glyoxylate cycle enzymes. When seeds were aged for 10 days, the positive effect of hormone priming on glyoxylate cycle enzymes was the greatest. Activities of Isocitrate lyase (Fig. 1) and Malate synthase (Fig. 2) in poorest vigor seed lot (40 days deteriorated seeds) were increased 22 and 21per cent, respectively, due to hormone-priming.

Singh and Amritphale [9] reported increased vigor of artificially and naturally aged seeds of soybean through application of GA3 and benzyladenine. In accordance with our results, Jeng and Sung [10] also found that glyoxylate cycle enzymes (isocitrate lyase and malate synthase) were increased by priming.

Table 1: Effect of deterioration time (days) and hormone priming on the germination traits of Persian silk tree (*Albizia julibrissin* Durazz.)

Deterioration (days)	Hormon	GP (%)	GR (seed number day ⁻¹)	MGT (day)	SRUR (mg seed ⁻¹)	SRUE(mg mg ⁻¹)	FUSR(mg mg ⁻¹)
Control	control	83.5 b	5.2 b	0.24 cd	0.008 b	1.3 b	0.64 b
	GA3	96.7 a	8.4 a	0.12 e	0.013 a	1.8 a	0.89 a
	SA	95.4 a	8.1 a	0.13 e	0.01 a	1.7 a	0.86 a
10	control	72.3 c	4.4 bc	0.28 cd	0.0055 c	0.95 de	0.56 bc
	GA3	87.6 b	5.5 b	0.22 d	0.0082 b	1.4 b	0.67 b
	SA	85.3 b	5.4 b	0.25 cd	0.0081 b	1.35 b	0.65 b
20	control	58.1 d	3.6 bc	0.3 bc	0.0042 d	0.82 ef	0.45 d
	GA3	67.8 cd	4 bc	0.27 cd	0.0051 c	0.89 de	0.52 cd
	SA	66.3 cd	3.9 bc	0.27 cd	0.0047 c	0.86 de	0.5 cd
30	control	40.4 f	2.6 d	0.36 b	0.0029 e	0.68 f	0.34 e
	GA3	52.1 e	3.3 cd	0.32 bc	0.0038 d	0.77 ef	0.4 d
	SA	52.2 e	3.3 cd	0.32 bc	0.0037 d	0.76 ef	0.4 d
40	control	23.5 i	1.4 e	0.73 a	0.0017 f	0.42 g	0.25 e
	GA3	38.7 f	2.4 d	0.4 b	0.0021 e	0.62 f	0.31 e
	SA	37.4 f	2.3 d	0.42 b	0.002 e	0.6 f	0.3 e

Different letters at each column indicate significant differences at 5% probability level by Duncan's multiple range test. GP: germination percent. GR: germination rate. MGT: mean of germination time. SRUR: Seed reservoirs using rate. SRUE: seed reservoirs using efficiency. FUSR: fraction of utilized seed reservoirs

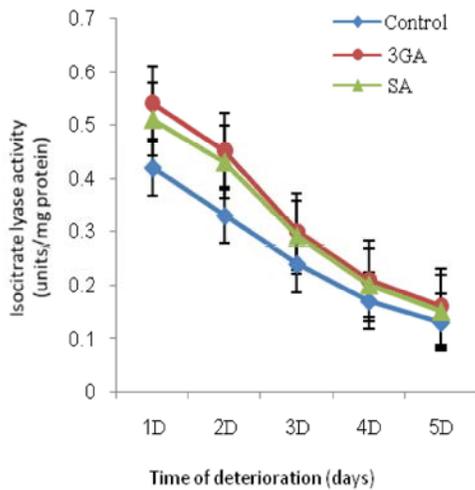


Fig. 1: Interaction of different vigor seed lots of Persian silk tree (*Albizia julibrissin* Durazz.) and hormone priming on the Isocitrate lyase activity. Bars indicate the standard deviation of data series

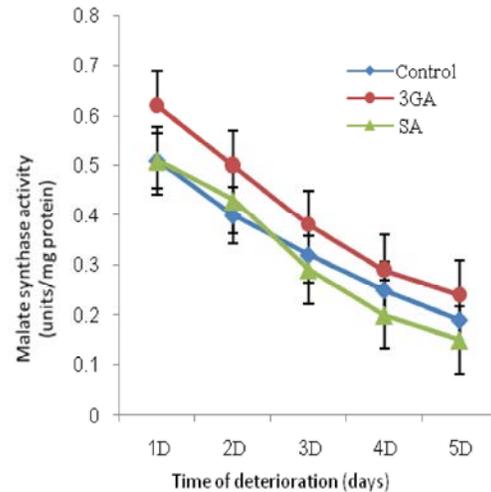


Fig. 2: Interaction of different vigor seed lots of Persian silk tree (*Albizia julibrissin* Durazz.) and hormone priming on the Malate synthase activity. Bars indicate the standard deviation of data series

Priming can partially improve the germination capability of deteriorated poor quality seed lots via some physiological and metabolic repairs within the seed. One of these is hormone priming. Perhaps, this type of pretreatment was led to enhanced glyoxylate cycle activity and then producing sugar from lipid (gluconeogenesis) and consequently reducing lipid peroxidation. Therefore, the germination capability of seeds and seedling establishment in 'Persian silk tree'

would be improved by invigorative effects of plant growth substances.

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