Pak. J. Bot., 46(6): 2131-2133, 2014.

RESPONSE OF *VITIS VINIFERA* L. SEEDS TO 22(S), 23(S)-HOMOBRASSINOLIDE

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Abstract

In order to determine effects of brassinosteroid on germination, seeds of var. Karasakız (*Vitis vinifera* L.) were soaked in different concentrations of 22(S), 23(S)- homobrassinolide solutions (0.025, 0.010, and 0.005 mgL^{-1}) followed by stratification for 30, 45, 60 and 90 days at 4°C in perlite. In comparison, seeds were also treated with gibberellic acid concentrations (0, 500, 1000, and 1500 mgL^{-1}). Seeds were sown in plastic cell containers containing perlite: peat moss: cocopeat (1:1:1 v/v) and the containers were placed under glasshouse conditions. Germination was not influenced by the brassinosteroid application. As the stratification time prolonged the germination increased. Effect of brassinosteroid on germination was not statistically important. However, it seemed that treatment with low concentration of the brassinosteroid resulted in similar germination percentage with the seeds stratified at least for 60 days.

Key words: Grapevine, Seed, Germination, Brassinosteroid, Stratification.

Introduction

Seed germination is obviously a complex event, which involves many metabolic processes where hormones play a very significant role (Ali *et al.,* 2005). Increasing germination in grape seeds has been a topic under extensive research. Different treatments and chemicals have been used to determine the best application that results in higher germination. Gibberellic acid, auxins, hydrogen cyanamide, thiourea, or calcium cyanamide are among the chemicals frequently tested either alone or in combination with temperature, scarification, or stratification experiments (Zhuo *et al.,* 1995).

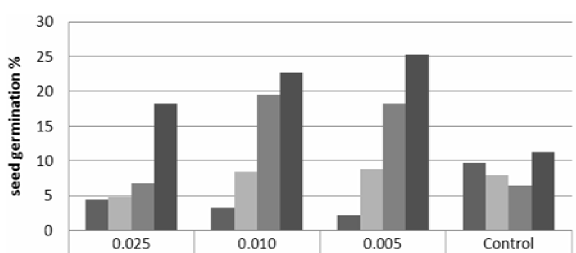
Brassinosteroids are a group of plant hormones with significant growth-promoting activities, which include seed germination, rhizogenesis, flowering, senescence, abscission, and maturation (Rao *et al.,* 2002). The ability of brassinosteroids to promote seed germination was observed in barley (Gregory, 1981), *Eucalyptus* (Sasse *et al*., 1995), tomato (Vardhini & Rao, 2000), tobacco (Leubner-Metzger, 2001), wheat (Hayat & Ahmad, 2003), and *Brassica juncea* (Sirhindi *et al*., 2009). Use of brassinosteroid compounds in grapes seed germination is relatively new research area. Keeping this in mind, this experiment was undertaken to assess the response of grape seeds to a synthetic brassinosteroid compound, 22S, 23S- homobrassinolide (SSHB).

Materials and Methods

The seeds were obtained from the island of Bozcaada (Tenedos) of Turkey where cv. 'Karasakiz' (syn. Kuntra) is commonly grown. Seeds were first surface sterilized with 0.5% (v/v) sodium hypochloride solution for 5 min. and washed several times with sterile distilled water. Then the seeds including the control group are treated with a fungicide for 30 min before stratification in sterile perlite at 4°C for 30, 45, 60, and 90 days. Every treatment consisted of four replicates with 100 seeds.

Stratified seeds were removed from the perlite and cleaned with sterile water and soaked for 24 h in four different concentrations (0.000, 0.005, 0.010, and 0.025 mgL^{-1}) of SSHB (Sigma, St. Louis, MO, USA) chosen arbitrarily and gibberellic acid-GA_3 (500, 1000 and 1500 mgL^{-1}). Control seeds for each compound were soaked in distilled water. At the end of 24 h, seeds were re-treated with a fungicide for 30 min and later directly sown under greenhouse conditions into the plastic cell containers filled with a growing medium composed of perlite: peat moss: cocopeat (1:1:1 v/v). Mean temperature and relative humidity throughout the experiment were 22°C and 67%, respectively. The containers were monitored weekly for about 10 weeks for the presence of germinated seeds with at least 1 mm root elongation.

The data were arcsine square root transformed and later tested with two-way ANOVA (stratification duration and concentration of the substances). Mean differences were tested with Duncan's multiple range test.



	0.025	0.010	0.005	Control
30 d	4,50	3,25	2,25	9,75
45 d	4,75	8,50	8,75	8,00
60 d	6,75	19,5	18,25	6,50
90 d	18,25	22,75	25,25	11,25

Fig. 1. Germination percentage (%) of *Vitis vinifera* L. var. 'Karasakiz' seeds stratified for different periods and soaked in different concentrations of 22(S), 23(S)-homobrassinolide (mgL^{-1}) solutions.

Results

Results showed that germination was under the influence of stratification time ($p \leq 0.01$) and 22(S), 23(S)-homobrassinolide concentrations ($p \leq 0.10$) (Fig 1). The lowest germination was obtained in the seeds stratified for 30 days (4.94%). Germination increased from 7.5% to 19.38% as the concentration was elevated. The highest concentration of the substance (0.025 mgL^{-1}) resulted in the similar percentage of germination as the control group. Lower concentrations on the other hand provided better germination. Although an interaction between the stratification time and the concentration of the substance, it seemed that medium and low concentrations provided a germination percentage at a similar level in the seeds stratified for 60 days compared with the highest concentration and the control.

Germination percentage of the seeds treated with gibberellic acid solutions (Table 1) showed that as the stratification period was longer than 60 days, statistically more seeds germinated. The effect of the compound in the 500 and 1000 mgL^{-1}concentration on germination was more clearly visible in the seeds stratified for 90 days. When the seeds were soaked in 1500 mgL^{-1} GA_3 solution, the difference between 60- and 90-day stratification periods disappeared.

Table 1. Germination ratios (%) of *Vitis vinifera* L. var. 'Karasakiz' seeds stratified for different periods and soaked in different concentrations of gibberellic acid solutions.

	Stratification period (day)			
GA_3 (mgL^{-1})	30	45	60	90
500	10.31Ab	11.41Ab	34.15Ba	58.09Aa
1000	17.84Ac	17.45Ac	42.72Ab	72.13Aa
1500	19.52Ab	18.26Ab	56.18Aa	69.17Aa
Control	9.85Ab	13.12Ab	37.46ABa	46.92Ba

*ANOVA to compare data (p<= 0.05): values sharing the same capital letter within a column and the small letter within a row are not significantly different

Discussion

Vitis vinifera L., seeds generally germinate low until the dormancy is broken by stratifying the seeds in cold for three or four months (Ellis *et al.*, 1983). Grape seeds with their thick seed coat present a mechanical barrier to germination (Conner, 2008). Treatment of seeds with different growth regulators and chemicals has been widely explored in order to increase germination of grape seeds (Zhou *et al.,* 1995) with varying, generally modest, success depending on cultivar, application time and rate of substances. This experiment investigated the effects of a type of brassinosteroid as well as gibberellic acid on germination of *V. vinifera* seeds.

Germination in the present study was not induced by combined effect of the length of stratification and the concentration of the substance used. Longer periods of stratification generally provided better germination in 'Karasakız' seeds. Conner (2008) tested the effects of hydrogen peroxide and gibberellic acid on the germination of the seeds stratified between 30 to 90 days and reported that 90-d stratification period gave the best germination in 'Fry' Muscadine seeds. Our finding that most seeds were germinated in the 90-day stratification treatment was supported. No clear effect of 22(S), 23(S)-homobrassinolide was determined. It appears that stratification period has the main influence on promoting germination of the 'Karasakız' seeds. Brassinosteroids were also found to be non-inductive of germination of wild Arabidopsis (Steber & McCourt 2001) and cress (Jones-Held *et al*., 1996).

The success of the 22(S), 23(S)-homobrassinolide in promoting germination of the grape seeds was not found close to the success of gibberellic acid, a growth regulator commonly used for germination induction. On the other hand, with the current results of the study, whether it is safe to conclude that brassinosteroids could be used in promoting germination in grape seeds is not clear. The benefit of using these compounds as a routine, being more expensive and less availability in the market is yet to be validated. As any of the growth regulating compounds, the activity of brassinosteroids differs with plant and type of the compound. The appropriate phase of application and concentration range in promoting germination should be seriously considered.

There is a long way ahead of the possible uses of brassinosteroids in the relatively new field of research area in grape growing. They act largely post embryonically with eminent effects on general plant growth through cell elongation, vascular differentiation and reproductive development (Depuydt & Hardtke 2011). Elucidating its effects on increasing seed germination would help breeders to gather as many seedlings as possible after a hybridization experiment. Pretreatment with brassinosteroids before stratification might result in shortening the stratification period. Soaking seeds for various periods, using intact or nicked seeds, different temperature regimes and preferably lower concentration ranges would provide scientific results. It is also suggested that following experiments should be designed with the treatments with proper ranges of both stratification time and compound concentration, as it may be even better with a lower concentration according the trend of the experimental results of this experiment.

Acknowledgement

This study has been submitted as a poster to the 8th National Viticulture and Technology Symposium held in Konya/Turkey between September 25 and 28, 2013.

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(Received for publication 18 July 2013)