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Short communication

Seed germination studies on cactus pear (*Opuntia* sp.)

R.A. Kaushik*, D. Pelah and Y. Mizrahi
The Institutes for Applied Research, Ben Gurion University of the Negev, Beer Sheva, Israel

Cactus pear – the common name for various species of the genus *Opuntia* (L.) Mill – is extensively grown as food and animal feed crops in many parts of the world (Barbera, 2). Plants of this genus grow particularly well under arid and semi-arid conditions. Because of the simplicity of vegetative propagation by stem cuttings, this method has been widely adopted for establishing commercial orchards. Yet, seed propagation is still used as a specialized tool for breeding purposes and for the propagation of pathogen-free plant material. Cactus pear seeds contain apomictic embryos, and thus propagation by seed may yield true-to-the-type offspring, which can be used for virus cleaning and rejuvenation (Mondragon-Jacoba, 8).

The cactus pear seed has a hard-lignified coat that serves to protect it from adverse environmental factors, but it also inhibits germination (Potter *et al.*, 10). A number of methods have been applied to attenuate this inhibitory effect, such as scarification or imbibition in hot water or concentrated sulphuric acid, but only with partial success (Godinez-Alvarez and Valiente-Banuet, 4; Potter *et al.*, 10; Mondragon-Jacobo, 8). Seed dormancy-both innate and enforced-is another strategy that enables cactus seeds to survive in harsh environments until conditions become favourable for germination. One of the causes of innate dormancy in cactus seeds is embryo immaturity, as has been demonstrated for *O. rastrera* seeds (Mandujano *et al.*, 7). Enforced dormancy, which is regulated by environmental conditions including light and temperature, has also been found in cactus seeds (Rojas-Arechiga *et al.*, 13; Rojas-Arechiga and Vazquez-Yanes, 11; Romero-Schmidt *et al.*, 12.)

Growth regulators have been applied to break seed dormancy and to synchronize seed germination in many plant species (Koorneef *et al.*, 5.) The effect of the application of exogenous gibberellic acid (GA_3) to seeds of different species of cactus varies from species to species, i.e., in some, it is an obligatory requirement for germination (Deno, 3) in others, it has no effect; while in yet other species, and it improves germination. In cacti, the effect of temperature on germination varies from species to species: some require fluctuating diurnal temperatures, while others germinate successfully at constant temperature. The latter may range from 17 to 34ºC, with an optimal value of 25ºC for a number of species (Nobel, 9). The present study was designed to examine the effect of plant growth substances, of nitrogenous compounds and of temperature (fluctuating versus constant) on the germination of the seeds of commercially important cactus pear species.

Seeds of the following species and clones of *Opuntia* were obtained from fruits collected from the experimental plot at the Institutes for Applied Research, Ben-Gurion University of the Negev, Beer-Sheva, Israel: *O. vulgaris, O. streptacantha, O. robusta, O. ficus-indica*cv. Gialla and *Oficus-indica* cv. Milpha alta. Seeds of *O. ficus-indica* cv. Ofer were collected from a commercial orchard. Some seeds of the latter cultivar were obtained from fully ripe fruits that had developed during the winter, while others were collected from fruits that had developed during the summer. The experiments were conducted in the winters (December-January) and autumns (September-October). Two identical experiments were conducted at four different temperature regimes, i.e., constant temperatures of 24 ± 1ºC and 31 ± 1ºC (in a laboratory incubator) and ambient temperatures of 27.5 ± 3.5ºC (in laboratory) and 26 ± 7.0 ºC (in a greenhouse). Viability of seeds was tested prior to sowing by applying the 2, 3, 5-triphenyl-tetrazolium chloride (TTC) test, as described by Aggrawal *et al.* (1).

Stock solutions of all chemicals were made up in water or ethanol, as relevant, and diluted with distilled water before use. Seeds were first soaked in tap water, and empty seeds, i.e., those that floated, were discarded. Only the dark brown uniform seeds that sunk were used for the experiments. The following soaking solutions were used: GA_3 (500 or 1000 ppm), thiourea (250 or 500 ppm), ethrel (250 or 500 ppm) and 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) (250 or 500 ppm). For each solution, 60 seeds were placed in a beaker containing 20 ml of the solution for 24 h at 27.5 ± 3.5ºC in the dark. Thereafter, the seeds were dried and subsequently sown on moist filter paper in glass dishes for the laboratory experiments or in a mixture of peat, perlite and vermiculite (1:1:1 v/v) for the greenhouse

*Corresponding author's present address: Department of Horticulture, Rajasthan College of Agriculture, Maharana Pratap University of Agricultue and Technology, Udaipur 313001, Rajasthan; E-mail Kaushik_ra@yahoo.co.in

experiments. The experiment was laid out in CRD and replicated thrice. Temperature and relative humidity were recorded during the experiments. Accumulated germination was monitored (in absolute values) after four weeks. Absolute values of germination were calculated as follows:

[100% viability x actual germination (%)]/[actual viability] = absolute germination (%).

Data are presented as an analysis of variance using the Tukey-Kramer test.

In the present study, the TTC test showed that various *Opuntia* species have different percentages of seed viability: high values were obtained for the seeds of *O. ficus-indica* cv. Gialla (95%), *O. streptacanthal* (95%) and *O. ficus-indica* cv. Ofer (92%), followed by *O. ficus-indica* cv. Milpa alta (80%) and *O. vulgaris* (55%). The seeds of *O. robusts* were the least viable (40%). Although only a few studies have evaluated the viability of cactus seeds, the data presented here clearly show that this is one of the major factors that should be taken into consideration when comparing seed germination between and within cultivars or species of cactus. A common cause of low germination in *Opuntia* seeds is impaired development or degeneration of the embryo. Primary dormancy may result from immaturity of the embryo, and in such a case the seeds require an after-ripeing period to germinate. Although it has been found for *O. rastrera* (Mandujano *et al.*, 7) and other species of cactus that germination improves as the seeds age (Rojas-Arechiga and Vazquez-Yanes, 11), our findings for *O. ficus-indica* cv. Ofer indicated that germination did not increase for seeds that were several months old and that viability decreased and rates of germination were poor (~25%) for two-year-old seeds

The results of germination experiments, under greenhouse conditions (alternating temperatures of 26.5 ± 7 ºC) for seeds of summer-harvested *O. ficus-indica* cv. Ofer, show that treatment with GA_3 or ethrel broke the endogenous dormancy and accelerated germination (Fig. 1). Maximum germination (73.33%) was obtained for seeds treated with 1,000 ppm of GA_3 the percentage being threefold higher than that for untreated seeds. Increased germination – but to a lesser extent – was also obtained with 500 ppm of GA_3. Application of GA_3 (both 500 and 1,000 ppm) also reduced the time required for germination by more than one week. In GA-treated seeds, cotyledons appeared on 10 and 12 days for seeds treated with 500 and 1,000 ppm, respectively, after initiation of the experiment in comparison with more than 20 days in untreated seeds or seeds subjected to other treatments. When 1,000 ppm GA_3 was applied to seeds collected from fruits that had developed out of season, i.e., during the winter, the maximum germination rate was 10%. Therefore, experiments of the second year were conducted only on seeds collected from fruits that had developed during the summer.

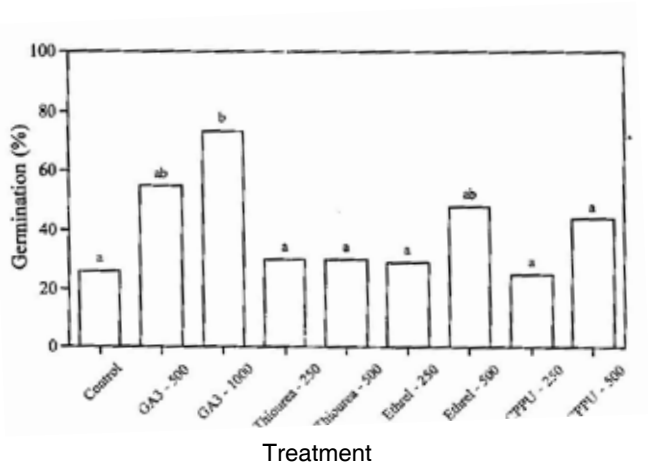

Fig.1. Effect of plant growth regulators and thiourea on germination of *Oputia ficus-indica* cv. Ofer.

Other plant growth regulators known to induce germination, such as ethrel (250 ppm) and CPPU (250 and 500 ppm), did not have significant influence on germination. Similarly, thiourea (250 and 500 ppm), as a source of nitrogen, did not influenced germination (Fig. 1), although it has been reported that nitrogen can induce germination of dormant seeds in a wide range of plant species (MacIntyre, 9). The effect of other sources of nitrogen on the germination of *Opuntia* seeds should be examined in the future.

Subjecting seeds of *O. ficus-indica* cv. Ofer to a constant temperature (24° or 31°C) had a negative effect on germination (Fig. 2). Furthermore, application of GA_3 to seeds incubated at constant temperatures had no effect on germination. Incubating seeds at alternating temperatures of 27.5 ± 3.5ºC did induce germination (25%), even without GA_3 application, while application of 1,000 ppm of GA_3 enhanced germination further (75%). In their review of cactus seed germination, Rojas-Arechiga and Vazquez-Yanes (11) suggested that the effect of fluctuating temperatures on cactus seed germination might be species dependent, while other works did not show any significant effect of fluctuating temperatures on germination of seeds of different cacti (Potter *et al.*,10; Rojas-Arechiga *et al.*,13). Godinez-Alvarez and Valiente-Banuet (4) found differences in the germination of seeds of the cactus species *Ferocactus latispinus* var. *spiralis* and *Echinocactus platyacanthus* fa. *grandis* exposed to constant versus fluctuating temperatures. They observed a marked increase (two- to three-fold) in germination of the seeds exposed to fluctuating temperatures. Although, Potter *et al.* (10) reported that germination in some species of *Opuntia* did not respond to fluctuating temperatures, our results

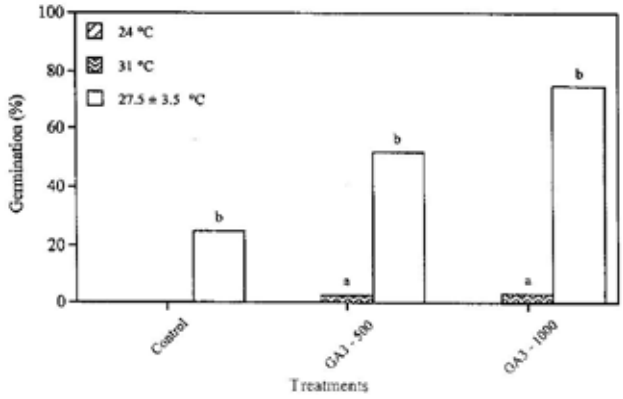

Fig. 2. Effect of constant vs. alternating temperatures on germination of seeds of *Oputia ficus-indica* cv. Ofer.

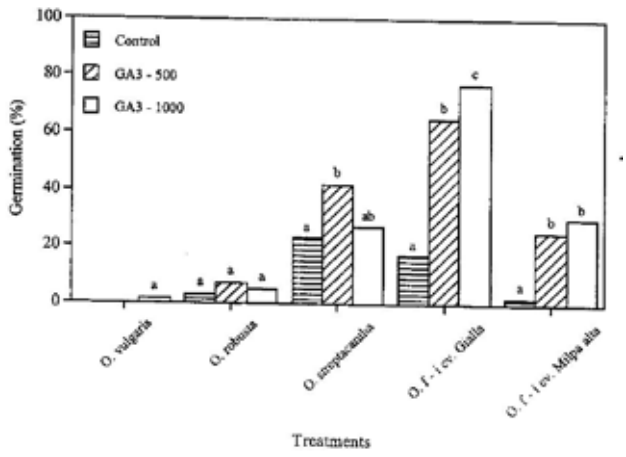

Fig. 3. Effect of treatment with GA_3 on seed germination of different species of *Oputia.*

show that for *O. ficus-indica* fluctuating temperatures do indeed enhance germination. In many of the experiments reported in the literature, the upper and lower limits of the range of temperatures fell between 20-35°C. In practical terms, it is likely that the high summer temperatures prevailing under field conditions are not suitable for commercial propagation by seeds since for most cactus species temperatures above 30°C are likely to be harmful to germination (Nobel, 9; Rojas-Areechiga *et al.*, 13). The requirement for fluctuating temperatures during germination may thus be interpreted as a physiological adaptation that promotes germination under the favourable conditions existing in the wet season when maximum daily temperatures are less than 30°C.

On the basis of the information generated by the experiments with *O. ficus-indica* cv. Ofer the efficacy of GA_3 application, 500 and 1,000 ppm, was tested in different species and clones exposed to alternating temperatures 26 ± 7°C in a greenhouse (Fig. 3). The most marked effect GA_3 application was found for *O. ficus-indica* cv. Gialla, with the differences in response of germination to the two different doses being statistically significant. In *O. streptacantha*, germination was promoted by 500 ppm of GA_3 and in *O. streptacantha*, germination was promoted by 500 ppm of GA_3 and in *O. ficus-indica* cv. Milpa alta, GA_3 had a positive effect at both concentration, the differences in response between the two not being statistically significant. In *O. vulgaris* and *O. robusta*, GA_3, application did not have a significant effect on germination. It is thus evident that the effect of GA application on germination of *Opuntia* seeds varied both within and between species.

The seeds of all the *Opuntia* species examined in this study exhibit endogenous dormancy, which inhibits germination. In *O. ficus-indica* cv. Ofer, application of GA_3 (and ethrel to a certain extent) in combination with alternating temperatures in the required range promoted germination and advanced the time of shoot emergence. Application of GA_3 in combination with alternating temperatures facilitated successful germination of *Opuntia* hybrid seeds. The findings that the effect of GA_3 application varies between and within species should be taken into account in breeding programms. Similarly, application of fluctuating temperatures during germination of *Opuntia* seeds should be used to promote germination (in cases in which it is inhibited by constant temperatures).

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