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Effect of Priming on Germination of *Lagenaria siceraria* Genotypes at Low Temperatures

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Abstract : This work was carried out to test whether germination of fifteen *Lagenaria siceraria* genotypes which were collected from western and southern parts of Turkey were improved by KNO_3 and NaCl treatments. The experiment was conducted in two consecutive years, 2004 and 2005. Germination tests were performed at 15 ^{0}C and 18 ^{0}C. Results indicated that the effect of the treatments varied between the genotypes, temperatures and solutions that were used. KNO_3 treatment was better and effective in larger number of lots than NaCl in both years. NaCl promoted germination in a few but inhibitive in large number of lots at both temperatures. As germination temperature was reduced, the positive effect of the KNO_3 became greater. As a conclusion, priming treatments, specifically KNO_3, appears to be promising in enhancing germination percentages of *Lagenaria siceraria* particularly at 15 ^{0}C that is the prevailing temperature in rootstock production for grafted watermelon seedling in unheated glasshouse conditions in early spring.

Key Words: *Lagenaria siceraria*, priming, germination, low temperature

Lagenaria siceraria Genotiplerinin Düşük Sıcaklıkta Çimlenmesi Üzerine Ekim Öncesi Uygulamaların Etkisi

Öz: Bu çalışmada; Türkiye'nin batı ve güney bölgelerinden toplanan, 15 adet *Lagenaria siceraria* genotipine ait tohumlar KNO_3 ve NaCl uygulamasına tabi tutularak çimlenme oranları araştırılmıştır. Araştırma, 2004-2005 yıllarında yürütülmüştür. Çimlendirme testleri, 15 ^{0}C ve 18 ^{0}C sıcaklılarda yapılmıştır. Sonuçlar, uygulamaların etkisinin genotipler, sıcaklıklar ve kullanılan solüsyonlar arasında fark olduğunu göstermiştir. Birçok genotipte her iki yılda da, KNO_3 uygulaması NaCl uygulamasına göre daha etkili ve iyi sonuç vermiştir. Her iki sıcaklıkta, NaCl az da olsa çimlenmeyi olumlu etkilemiştir; fakat, birçok genotipte çimlenmeyi engellemiştir. Çimlenme sıcaklığı düştükçe KNO_3 uygulamasının pozitif etkisi daha iyi gözlenmiştir. Sonuç olarak; uygulamalar, özellikle de KNO_3 uygulaması ile 15 ^{0}C sıcaklıkta, *Lagenaria siceraria* tohumlarının çimlenme yüzdesi arttırılmıştır. Belirtilen uygulama, erken ilkbahar döneminde ısıtmasız sera koşullarında aşılı karpuz fidesi için anaç üretiminde etkili şekilde kullanılabilir.

***A*nahtar Kelimeler:** *Lagenaria siceraria,* ekim öncesi uygulamalar, çimlenme, düşük sıcaklık

Introduction

Cultivated *Lagenaria siceraria* (Malign) Stanley is commonly known as the white-flowered bottle gourd. It has been cultivated annual monocious, vigorous climber species and five wild perennial diocious species. The genus *Lagenaria* also contains five wild species: *L. brefilora* (Benth) Roberty, *L. abyssinica* (Hook F.) Jeffrey, *L. rufa* (Gilg) Jeffrey, *L. spherical* (Sonder) Naudin and *L. guineensis* (G. Don) Jeffrey (Motimoto et al. 2005). The fruits are generally eaten as a vegetable in Africa and Asia. Immature fruits are eaten by boiling, frying or stuffing like fruit of *Cucurbita pepo*. The mature fruit is often scooped out and the skin used as containers, bowls, music instruments, decorative purposes or in some cases, fishing floats. Shoots, tendrils and leaves are also cooked and the seeds are removed for oil extraction or for use in cooking. Tendrils and young leaves are also used for some medical

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purposes (Tindall, 1983). Furthermore, *L. siceraria* is used as rootstocks for watermelon against soil-born diseases and low soil temperature. Grafting of watermelon onto bottle gourd was first performed in Korea and Japan in the late 1920s (Ashita 1927). *L. siceraria* is one of the species used as rootstock for watermelon and it shows high compatibility rate with watermelon (Lee 1994, Oda 1995; Yetisir and Sari 2003).

Watermelon seedlings are grafted for various purposes such as to control *Fusarium* wilt, to increase low temperature tolerance and yield by enhancing water and plant nutrient uptake (Masuda et al. 1981, Oda 1995). Although cucurbit species of *Cucurbita moschata*, *Cucurbita maxima*, *Benincasa hispida*, are commonly used as rootstocks in watermelon grafting was first performed on *Lagenaria* species and are still being used in a large extend. Most recent studies comparing various rootstocks indicated that *Lagenaria* type rootstocks produced higher yield and vigorous plants and are more resistant to *Fusarium* wilt compared to those cucurbits used in watermelon production (Yetisir and Sari 2003).

Watermelon has been cultivated intensively under low tunnels in southern part of Turkey for early production. One of the basic problems in grafted watermelon seedling production is erratic and slow germination of rootstock seeds (*Lagenaria* species) in early sowings (Shik et al. 1999). Rapidly developed rootstock seedlings will certainly provide earlier grafting due to the longer hypocotyls which will make early transplanting in the field possible thus resulting in early maturation. However, it was reported that *Lagenaria* species are very sensitive and require longer times to germinate at low temperatures (Chang et al. 1996) which are prevailing during the early spring sowings. This delays fast and efficient grafted seedling production.

Several priming treatments have been reported to enhance germination percentage and rate of seeds under low temperature sowings in various crop seeds (Bradford et al. 1990, Taylor et al. 1998, Lin and Sung, 2001; Demir and Mavi, 2004). Salt solutions have been used for such aims and particularly KNO_3 and KH_2PO_4 treatments were found promoting the germination percentage of gourd seeds at low temperatures (Shik et al. 1999, Chang et al. 1996). One important aspect of such treatments is that their effect may not only change due to the concentration, treatment period, chemical agent or treatment temperature but also according to genotypes and even among the lots within the same species (Heydecker and Coolbear 1977).

In this study, we tested whether KNO_3 and NaCl treatments can enhance low temperature germination percentages of fifteen *Lagenaria siceraria* genotypes which were collected from southern and western parts of Turkey.

Materials and Methods

Seed materials: This experiment was conducted on fifteen genotypes of *Lagenaria siceraria* collected from various regions of western and southern parts of Turkey. Experiment was conducted in two consecutive years in seed science laboratory at Ankara University. Genotypes were collected in 2004 (first year) and same lots were grown in 2005 (second year) in Central Horticultural Research Experiment Station in Mersin / Turkey to obtain seeds. Seeds were extracted from mature fruits (70-75 days after anthesis) dried at 25 ^{0}C. Seeds were hermetically stored at 5 ^{0}C until use. Seed moisture content determined by high temperature oven method (130 ^{0}C , 1 h) (Ista 1996) which were lower than 10 %.Genotypes number and codes were given along with 10 seeds weight, seed coat colour, seed length and seed width in Table 1. Every measurement was carried out on five replicates of 10 seeds selected randomly in each genotype and means were taken.

Advantage or disadvantage of the treatments compared to untreated seeds was presented in Table 3.

Salt priming treatment: Seed priming was carried out on top of filter paper (GmBH, Made in Germany) moistened with 30 ml of 4 % KNO_3 or 1 % NaCl solutions and kept at 20 ^{0}C for 6 days in the dark in sandwich boxes (18x9x5 cm) (Bradford 1985). Three hundred seeds were used for each priming solution and genotype. During the priming treatment, boxes were covered with plastic film to prevent loss of liquid. At the end of the treatment seeds were washed under tap water for 30 seconds and dried to the original weight on top of filter paper on laboratory bench (20±2 ^{0}C, 45-50 % RH) for two days. Germination tests were conducted within two days after the treatment and seeds were stored at 5 ^{0}C during that period.

Table 1. Changes in colour, length, width and 10 seed weights of seeds of genotypes of *Lagenaria spp*. used in this work

Genotypes	10 seeds weight (g)	Seed coat color	Seed length (cm)	Seed width (cm)
31-42 A	1.49	Pale yellow	1.47	0.46
46-12 B	2.63	Dark brown	1.98	0.79
07-34 C	2.07	Yellow	1.58	0.83
27-01 D	2.06	Pale brown	1.73	0.64
46-01 E	1.82	Pale grey	1.67	0.78
07-31 F	2.42	Dark brown	1.63	0.58
31-07 G	2.03	Tan	1.84	0.54
33-10 H	1.94	Grey	1.76	0.62
31-49 I	2.34	Pale brown	2.02	0.78
33-37 J	2.28	Pale brown	1.72	0.69
07-12 K	1.57	Dark yellow	1.49	0.63
46-11 L	1.96	Pale brown	1.06	0.56
07-14 M	2.61	Dark brown	1.97	0.67
01-13 N	1.36	Pale white	1.75	0.55
01-16 O	2.48	Pale grey	1.59	0.72

Table 2. The effect of KNO_3 and NaCl treatments on 4th and 7th day germination percentages *Lagenaria spp*. At 15°C.

	15°C											
	2004						2005					
	KNO_3		NaCl		Control		KNO_3		NaCl		Control	
Genotypes	4 day	7 day	4 day	7 day	4 day	7 day	4 day	7 day	4 day	7 day	4 day	7 day
36-42 A	99	99	4	5	1	1	58	86	16	28	0	0
46-12 B	95	99	37	67	1	27	35	72	4	11	0	4
07-34 C	61	92	7	20	8	63	30	63	8	9	0	4
27-01 D	99	100	17	21	47	87	0	61	7	12	0	0
46-01 E	56	59	0	1	4	37	40	55	31	35	0	11
07-31 F	3	28	1	28	0	33	16	52	11	21	0	23
31-07 G	96	100	19	25	0	3	8	45	5	16	0	0
33-10 H	93	96	0	96	0	1	33	41	4	4	0	0
31-49 I	75	77	1	4	3	5	29	35	3	3	1	0
33-37 J	73	85	8	29	15	52	11	35	0	3	0	3
07-12 K	40	57	1	3	16	56	15	23	12	17	13	28
46-11 L	47	71	1	0	1	24	1	20	0	0	0	0
07-14 M	39	47	0	0	0	3	3	19	1	7	1	7
01-13 N	3	3	0	0	0	5	0	1	0	1	0	0
01-16 O	27	69	12	24	1	21	0	0	0	0	0	0
Mean	60.4a*	72.1A	7.2b	21.5B	6.5b	27.9B	18.6a	40.5A	6.8 a	11.1B	0.9b	5.3B

* Small letters are to compare 4th day big letters are for 7th day germination among the treatments within the same year

Table 3. The advantages and disadvantages of the KNO_3 and NaCl treatments on germination of *Lagenaria spp*. At 15 and 18 °C in 2004 and 2005.

	2004				2005			
	KNO_3		NaCl		KNO_3		NaCl	
Genotypes	15^0C	18^0C	15^0C	18^0C	15^0C	18^0C	15^0C	18^0C
31-42 A	98	27	4	-56	87	34	28	12
46-12 B	72	1	40	-11	68	-1	7	-36
07-34 C	29	-3	-1	-47	59	11	5	-59
27-01 D	13	-1	-66	-45	61	-4	12	-39
46-01 E	22	15	-36	-60	46	-6	24	-12
07-31 F	-5	-5	-29	-33	29	-7	-2	-27
31-07 G	97	4	22	-52	45	-11	16	-49
33-10 H	95	19	-1	-73	41	61	4	-15
31-49 I	72	69	-1	-1	37	-3	3	-86
33-37 J	33	1	-23	-58	32	-12	0	-70
07-12 K	1	-10	-53	-86	-5	20	-11	-57
46-11 L	47	35	-24	-32	20	90	0	24
07-14 M	44	40	-3	-29	12	-3	0	-50
01-13 N	2	-32	-5	-89	1	7	1	1
01-16 O	48	-2	3	-16	0	22	0	-12
Total	668	158	-173	-688	533	198	87	-475

Low temperature germination tests Germination of treated and untreated seeds were carried out by using three replicates of fifty seeds each at 15 ^{0}C and 18 ^{0}C between wet rolled paper towels (ISTA, 1996). Fifty seeds were placed between three (two down one up) of 20x20 cm sized filter papers wetted with 18 ml of distilled water (EC 5 μScm^{-1} g^{-1}). Rolled papers were placed in self zipped and tightly closed polyethylene bags and kept in the dark at appropriate temperatures. Towel papers were weted by spraying the water as required throughout the germination period. Seeds with two mm radicle protrusion were considered to have germinated. Counts were made 4 and 7 days after the test was set up.

Means of the three replicates of the treated and untreated seeds of each genotype were compared by Duncan's multiple range test at 5 % level. Statistical analysis was carried out by using SPSS statistical package.

Results and Discussion

KNO_3 treatment significantly increased germination percentages of 5 lots at 18 ^{0}C and 12 lots at 15 ^{0}C ($P<0.05$) in 2004 (Figure 1). Only in one lot (lot N), KNO_3 was found adversely effective on germination. Contrastingly, NaCl treated seeds had lower germination than untreated ones in 12 lots at 18 ^{0}C and in 8 lots at 15 ^{0}C in 2004. NaCl was effective only in two lots, B and G at 15 ^{0}C, out of 15 lots and two temperatures.

Although all are not the same lots as in 2004, KNO_3 treatment enhanced germination of five lots significantly ($P<0.05$) at 18 ^{0}C in 2005 (Figure 2). However, its effect extended to 12 lots at 15 ^{0}C in the same year. Germination of control seeds of 10 lots was significantly higher than that of NaCl treated ones at 18 ^{0}C while only three treated lots had higher germination than control at 15 ^{0}C in 2005.

Fourth and seventh day counts of germination tests in Table 2 indicate that KNO_3 treated seed lots had much faster germination than both NaCl treated and control seed lots at 15 ^{0}C. Within the first four days of the test means of the 15 lots were 60.4 % and 18.5 % in KNO_3 treated ones in 2004 and 2005. Corresponding values in NaCl treated and control were 7.2 and 6.8 %, 6.5 % and 0.9 %, respectively, in two consecutive years (Table 2). Advancement observed by KNO_3 compared to NaCl and control on seventh day counts showed similar trend and was significantly ($P<0.05$) in favour of KNO_3 treatment (Table 2).

Total advantages of the two treatments in two consecutive years with regard to temperatures were presented in Table 3. The largest advantage was observed in KNO_3 treatment in both years and at temperatures.

At 15 ^{0}C KNO_3 increased total germination 668 in 2004, 533 in 2005. It decreased to 158 and 198 at 18 ^{0}C in 2004 and 2005, respectively. NaCl treatment was disadvantageous at both temperatures in 2004. Contrarily, it is advantageous at 15 ^{0}C but not at 18 ^{0}C (Table 3).

Priming seeds for germination under unfavourable temperatures reported in various crop seeds (Pill 1995). Following priming, seeds have completed phase 1 (hydration) and II (lag phase) of germination and only require a favourable water potential gradient for water uptake in order to begin radicle growth (Pill 1995). Specifically, salt-priming in which various salts were used were found enhancing germination at low temperatures in cucurbits (Sachs 1977, Bradford 1985, Nerson et al. 1985, Demir and Oztokat, 2003, Korkmaz et al. 2005). Shik et al. (1999) and Chang et al. (1996) indicated that KNO_3 was very promoting germination of *Lagenaria* seeds at low temperatures. Treated seeds had not only high germination percentages but also low mean germination time. It was reported that induction of KNO_3 in germination occurs through obtaining excessive O_2 and phosphate uptake (Bliss et al. 1986). Moreover, it is effective on dormancy release and suggested as a routine use in germination tests in those seeds likely to be dormant (ISTA 1996).

Advancement of priming may depend on various factors occur during the treatment. One hypothesis is that it can be due to metabolic repair of deleterious events during treatment and that advancement in germination events i.e. enzymatic changes, to reduce lag time between imbibition and radicle emergence does also occur (Bradford et al. 1990). Along with metabolic advancement, Nerson et al. (1985) found that KNO_3 priming also increased embryo length in tetraploid watermelon seeds. Treated seeds had stronger embryos and in turn broke up the seed coat blockage. We have not measured the embryonic development of the genotypes in our work but it is likely that priming may increase germination of some *Lagenaria* genotypes through increasing the embryo size. Hard seed coat is one cause of emergence failure in watermelon seeds (Sung and Chiu 1995). Priming might soften the seed coat and in turn ease of the mechanical restriction on embryo.

One of the clear findings of the present work is that NaCl treated seeds gave worse performance than

those of control seeds. Some of the previous studies had shown similar results. NaCl hinders DNA and RNA synthesis (Bliss et al. 1986) and Ca and K uptake during treatment (Durrant et al. 1983). It was also proposed that NaCl may not allow the seeds to reach the critical level of seed moisture so that metabolic processes (second phase of imbibition, lag phase) are not activate sufficiently (Ashraf and Foolad 2005). However, we did not determine the final seed moisture content that seeds reached following treatment. Therefore we are not able to compare two salttreatments with regard to that aspect. It was reported that critical moisture level or initial stage of lag phase (second part of imbibition) varies among the species and it is specifically related to chemical composition of seed (Vertucci 1989). This probably needs to be determined for *Lagenaria spp* to understand the starting point of activation of pre-sowing seed treatment mechanism.

Grafted vegetable seedling technology has improved greatly in recent years. *Lagenaria spp*. were used as rootstock widely in watermelon seedling production (Yetisir and Sari 2003). Obtaining fast and well developed rootstock seedlings at low temperatures are advantageous in early grafting which would lead to early maturation. As a conclusion, KNO_3 treatment has an advantage in obtaining well-developed rootstocks seedlings and can be a useful tool for achieving such aim.

Figure 1. Germination percentages of *Lagenaria spp*. genotypes at 15 and 18 °C in 2004

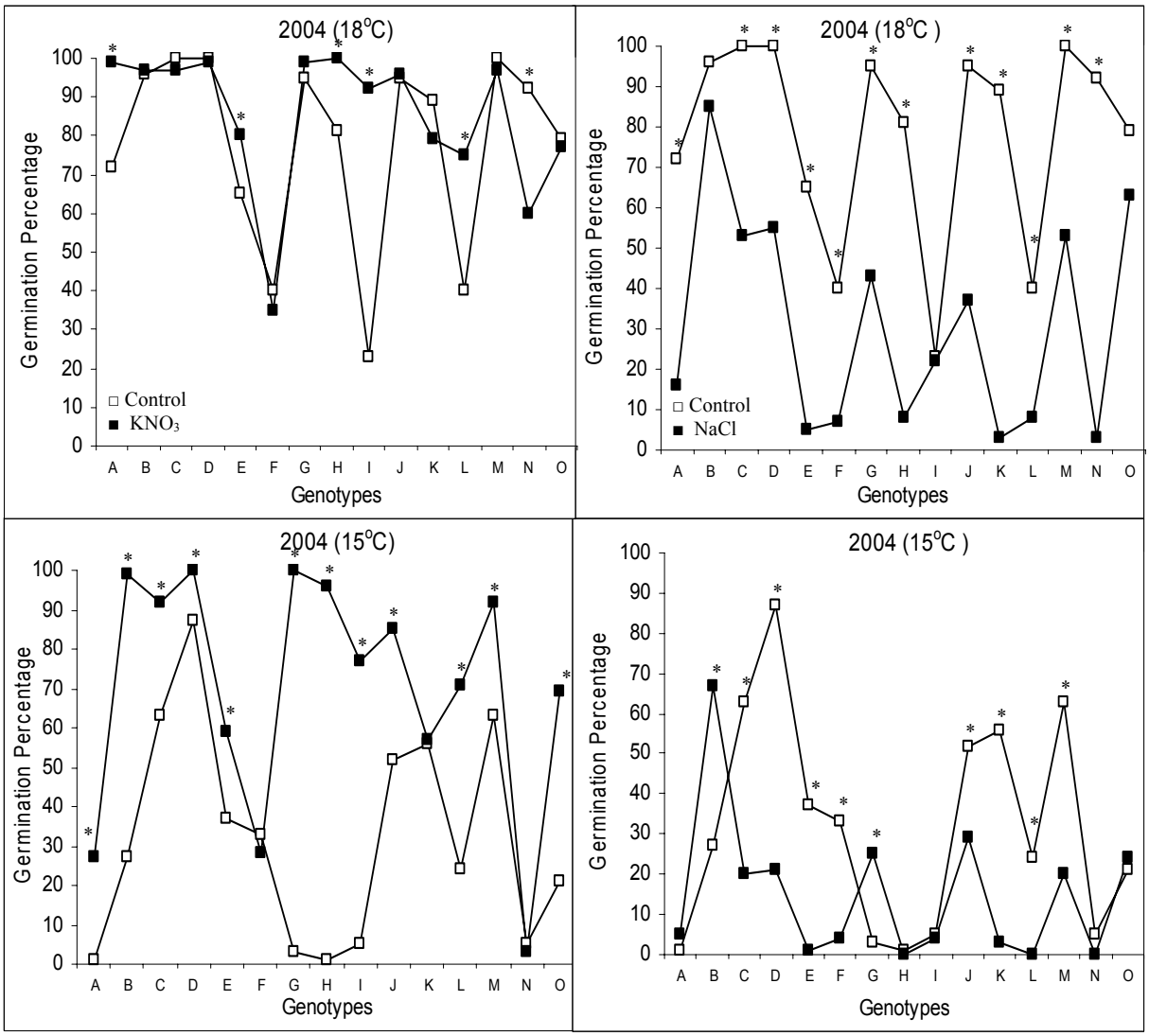
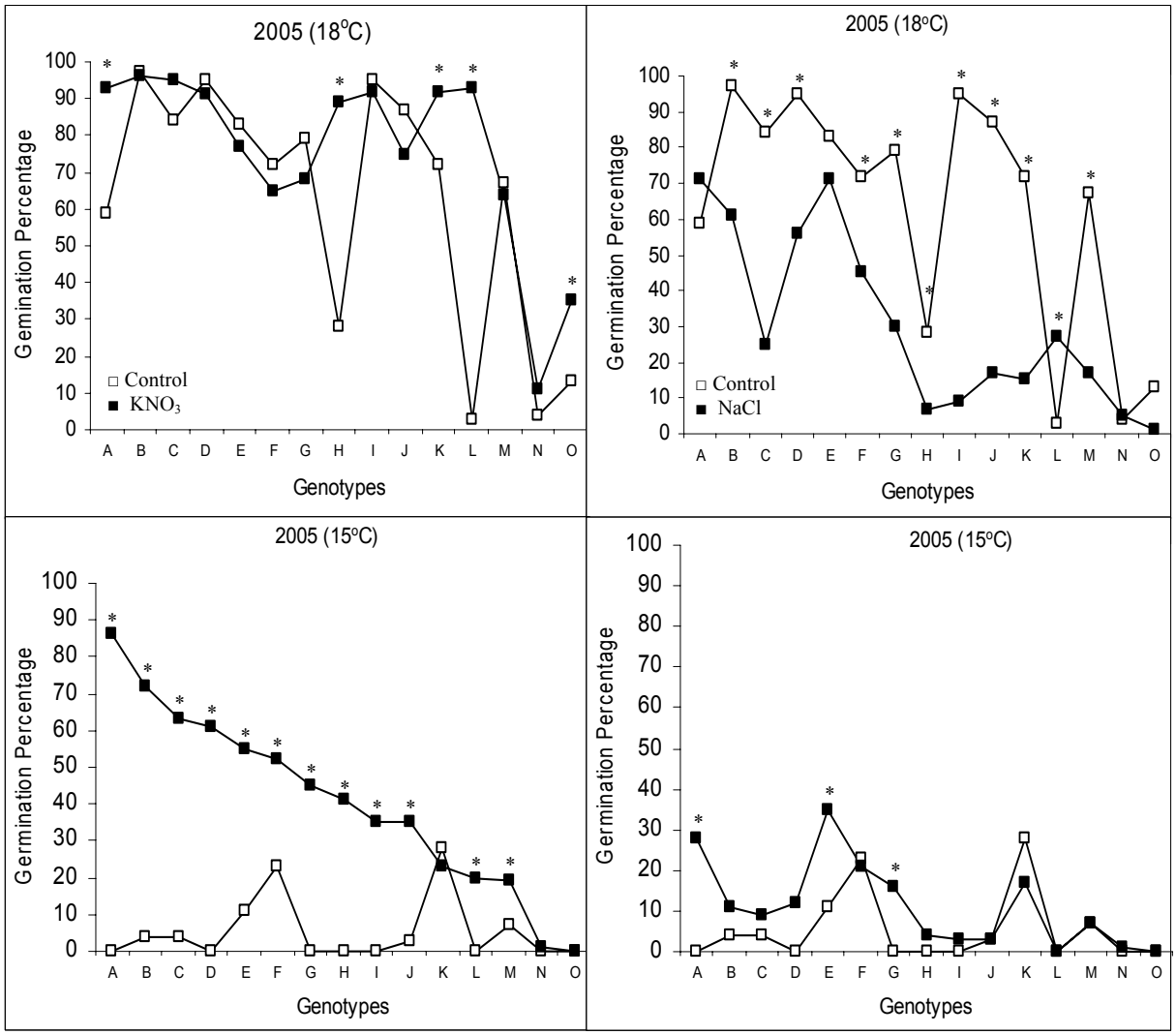


Figure 2. Germination percentages of *Lagenaria spp*. genotypes at 15 and 18 °C in 2005



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