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Priming techniques and size characteristics of triploid and tetraploid watermelon seed

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PRIMING TECHNIQUES AND SIZE CHARACTERISTICS OF TRIPLOID AND TETRAPLOID WATERMELON SEED

by

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ABSTRACT

Triploid watermelon seed, which produces seedless melons, are progeny of a 4Nx2N cross, which also produces 4N embryos by self-pollination of the 4N parent. The 3N and 4N seeds are not visually distinguishable, increasing costs to growers, who cannot market the low-quality 4N melons. Efforts were made to separate 3N and 4N seeds by thickness and weight. Means for the 3N and 4N groups in both cases were nearly equal.

Low viability of 3N seed requires transplanting. Seed 'priming' has been shown to improve germination in other crops, and would be an attractive method for seedless watermelon growers. Seeds from the open-pollinated 4Nx2N cross were primed in solutions of H_2O, polyethylene glycol '8000', and KNO_3, or left untreated. Treatment times were 1, 3, and 6d, and treated seeds were subsequently dried for either 1 or 7d. Seeds were scored for germination or emergence in the lab and for emergence under field conditions. H_2O was better than KNO_3 or PEG, but not always better than the control. 1d in treatment was superior to 3 or 6d, but length of drying time was insignificant. In the field trial, treatments did not differ in emergence.

INTRODUCTION

Watermelon [Citrullus lanatus (Thunb.) Matsumi and Nakai] originated in Africa (Shimotsuma, 1960) but is distributed today throughout Egypt, tropical Africa, and south, west and central Asia. Classification of watermelons in the literature has led to a slightly confusing array of names. In addition to C. lanatus, it has been referred to as C. vulgaris and Colocynthis citrullus. Successful crosses between C. colocynthis and C. vulgaris were obtained by Shimotsuma (1958) and by Whitaker (1933). Shimotsuma (1958) reported that the fruits obtained from reciprocal crosses were normally developed and produced viable seed, chromosomes in the pollen mother cells exhibited normal pairing, and hybrid vigor was apparent in the F_1 hybrids. Whitaker (1933) suggested that C. colocynthis is the parent of C. vulgaris, supporting his argument with the following reasons: 1) crosses between C. vulgaris and C. colocynthis are easily obtained, 2) the haploid number of chromosomes is 11 in both species, 3) F_1 hybrids exhibit fertility, and 4) both species are endemic to the same geographical region.

The growth habit of watermelon is annual, and it prefers sandy loam soil and hot temperatures (25° to

40°C). Flowers are monoecious with staminate flowers appearing on the plant earlier than pistillate flowers. There are usually 7 staminate to 1 pistillate flowers (Whitaker and Davis,1962). The flesh may be red, orange, yellow or white, and may be sweet or bitter, with some gradation present. The chromosome number in *C. lanatus* has been confirmed as 2N=2X=22 (Shimotsuma, 1958; Whitaker, 1930).

Triploid watermelons are theoretically seedless and result from the cross between a tetraploid (4N) female and a diploid (2N) male. The reciprocal cross has been reported to be unsuccessful (Kihara, 1951). An early effort to produce diploid seedless melons involved the treatment of diploid seeds with colchicine and flowers on resulting plants with naphthalene acetic acid (Wong, 1941).

Today, tetraploids are produced by treating diploid seedlings with colchicine. These are placed in a crossing block with diploid plants, with bees as the primary pollinators. The resultant open-pollinated seed can be either tetraploid, due to self-pollination, or triploid due to pollination by a diploid. Triploid and tetraploid fruit are distinguished by use of a genetic marker for rind color (Wall, 1960). The tetraploids,

used as the female parent, are light green, while the diploid pollinators are dark green. Resultant tetraploids from self-pollination are light green, but triploids have dark green stripes on a light green background. In Taiwan and Japan the 4N x 2N crosses are made by hand-pollination, but this method is not used in the U.S. since labor costs would be prohibitive. Still, in the U.S., this open-pollinated seed carries a high price -- up to 20 times that of the diploid varieties. (Burrell, 1988).

The seedless characteristic of triploid fruit is theoretically due to the low probability of forming gametes with balanced chromosome numbers. The probability of a balanced embryo, either diploid or triploid, would be 1/1024, or about 0.1%. However, a high proportion of developed seeds have been obtained from triploid fruit. These have been germinated and many of the resulting progeny were shown to be trisomics (D.T. Ray, unpublished). Thus, if trisomic embryos are also viable, the theoretical number of seed expected in the so-called seedless watermelon are increased.

The primary tetraploids developed for use in triploid seed production in the U.S. were not selected for disease resistance or other desired characteristics

(Eigsti, 1979), but extensive breeding has been undertaken in Japan (Shimotsuma, 1961). For example, 4N x 2N crosses were made for characteristics such as exocarp pattern, rind thickness, time to maturity, flesh texture, percent sugar content, mean yield, and mean fruit weight.

Tetraploid and triploid seed coats are thicker than the diploid and have one or more distinct fissures. The triploid embryos often do not fill the seed coat and their cotyledons are often unequally developed. Tetraploid embryos are more developed in relation to triploids, but are not as developed as diploids (Kihara, 1951).

There are cultural disadvantages to triploid plants because early in the season they grow more slowly than diploids, until about six weeks, when they start to grow more rapidly and surpass the diploids (Kihara,1951). Also, since pollen on the triploid plants is sterile, diploid pollinators must be interplanted to stimulate triploid fruit development.

Other problems of triploid watermelons are: triploid seeds do not germinate in cold, wet soils as well as diploids; germination is slower due to the reduced size of the embryo and the thicker seed coat;

fissures on the seed coat provide safe harbour for fungal spores; triploid seedlings will not tolerate high soil moisture conditions between seedling stage and runner development; and, fruit set on triploid plants is later than most diploid cultivars (Partridge, 1979). Because of these problems triploid seeds cannot be direct-seeded, but seedlings must be transplanted.

The purpose of this study was three-pronged: 1) establish germination protocol and determine a base rate of germination for polyploid watermelon seed; 2) test for seed size differences between populations of triploid and tetraploid seed; and 3) explore seed priming techniques that could lead to improved germination rates of polyploid watermelon seed.

LITERATURE REVIEW

Separating Watermelon Seeds by Size

A sorting method was developed by Shimotsuma and Matsumoto (1957) to distinguish triploid from tetraploid watermelon seed based on weight and seed thickness. Root tip cytology was used to confirm their findings, and in general they were able to attain success. They found that triploid seeds were thinner and lighter than tetraploid seeds, but both were thicker and heavier than diploid seeds. Length and width were found to be similar.

Effects of Osmotic Pre-treatment on Germination

Seed germination is described as a process of embryo imbibition and radicle emergence from the seed coat. Water and oxygen are the only substances taken up by seeds during germination. During this process, three important functions are being carried out: 1) breakdown of proteins, starches, and other materials in the seed, 2) transport of breakdown products from the endosperm to the embryo and from the cotyledons to the meristems, and 3) synthesis of new materials from the breakdown products formed (Mayer and Poljakoff-Mayber, 1963).

Protein is the major component of seeds that incorporates water. Other components, such as mucilages, cellulose and pectins also hydrate during imbibition. Starch, however, does not hydrate, even when it is present in large amounts (Mayer and Poljakoff-Mayber, 1963).

The pH of the solution affects the swelling of seeds, but, since proteins carry positive and negative charges, it is their isoelectric point that is critical to imbibition. At their isoelectric point, proteins imbibe the least. Beyond that in either direction, they imbibe greater amounts (Mayer and Poljakoff-Mayber, 1963).

In watermelon, seedling emergence follows imbibition as the cotyledons expand and true leaves develop. The radicle emerges and, subsequently, the hypocotyl and cotyledons emerge, the cotyledons open, and finally the true leaves develop.

Imbibition leads to physiological changes in the embryo which may be interrupted at some point before radicle emergence, but which is continued at a later time. But at some point these processes become irreversible (Heydecker, 1974). At imbibition, embryo cells experience a change in water potential from about

-1000 bars to about -10 bars (Heydecker and Gibbins, 1978). Simon and Mills (1983) suggest that so much force is created by the rapid entry of water that membrane rupture occurs. Putting the seeds in an osmotic solution would slow water entry into the seed/embryo, still allowing imbibition, but at a slower rate. However, some osmotica at -10 bars have been shown to completely inhibit germination (Heydecker, 1974), reaffirming the necessity of a differentially permeable membrane.

Many experiments have been conducted to evaluate the effects of osmotic pre-treatments (also called priming) on seed germination (de Klerk, 1986; Sachs, 1977; Cole and Wheeler; 1974). De Klerk (1986) and Sachs (1977) both found that priming undertaken at optimal temperatures for germination allowed subsequent germination at supraoptimal (de Klerk, 1986) or suboptimal (Sachs, 1977) temperatures. This supports the idea that pre-germinative processes are initiated sufficiently to allow radicle emergence when water potential is sufficient for uptake by the embryo. This idea is supported by Heydecker and Coolbear (1977), who maintain that low osmotic potentials may prevent the radicle from emerging while allowing initiation of some metabolic processes. But osmotic solutions may not

always be the best priming treatments. Nerson et al. (1985) found that water used as the priming medium on tetraploid watermelon seed, followed by drying, improved germination significantly.

Some non-ionic molecules, such as mannitol, glycerol, and glucose are not useful osmotica for seed pre-treatment to improve germination. This is partly because they are capable of entering seed tissues (Manohar, 1966). PEG '6000' (Sigma Chemical Company) and other polymers with a molecular weight greater than 4000 inhibit oxygen flow into the seeds. While PEG is a physiologically inert osmoticum, oxygen solubility and mobility are severely reduced (Heydecker and Coolbear, 1977). Osmotica found useful in seed priming include KNO_3 and K_3PO_4 (Sachs,1977). While these salts probably do not contribute nutrients to the developing embryo (Heydecker and Coolbear, 1977), they work well as osmotica, and do not restrict water movement or solubility as PEG does.

Problems in Seedless Watermelon Production

While seed priming techniques have been shown to improve germination of polyploid watermelon seeds (Sachs, 1977), this method is not yet economically viable. Incorporation of a priming technique in the

industry could eliminate the need to transplant seedlings. Cost factors must be considered, but should be favorable for transfer to this method of cultivation. Still, there are all the other problems of seedless watermelon production. Of primary concern is the mix of 3N and 4N progeny seed. While Shimotsuma and Matsumoto (1957) were able to separate 3N and 4N seeds by thickness, growers today are experiencing up to 30% 4N seed mixed in with their 3N seed (D.T.Ray, unpublished). This may be due to inadvertant selection for thinner seed coat in the 4N seed.

MATERIALS AND METHODS

Germination Tests of 'Tri x 313' Watermelon Seed

Germination tests were conducted with 'Tri x 313' seed obtained from Perry Nelson of Viking Agro-Industries, Phoenix, Az. The seeds had been passed through screens, segregating them into five groups: <2.2mm, 2.2 to 2.4mm, 2.4 to 2.6mm, 2.6 to 2.8 and >2.8mm. Controls used in the test were the diploid varieties Charleston Gray (CG) and Charleston Gray No. 5 (CG5). These were obtained from Hollar Seed Company.

Seeds were placed in 100x15mm petri dishes, with 100 seeds of each size placed in 4 dishes, 25 seeds per dish. A Whatman #1 filter paper was placed on the bottom of the petri dish, and a second one was used to cover the seeds. After the top filter paper had been placed in the dish, 13ml distilled water was added and the dish covered. Seeds were germinated in a germinator set at 30°C. Tests were allowed to run until no more seeds germinated. This usually took 1 to 2 weeks. Each run was a replication, with a total of four replications. Extremely low germination results for the polyploid seeds led to the decision to discontinue using seeds from this particular seedlot for further experimentation.

Evaluation of Seed Thickness

Open-pollinated seed from a 4N x 2N cross were obtained from Herb Partridge of the Munday Vegetable Growers Co-op, Munday, Texas. A hand-held Manostat with accuracy to the 0.1mm was used to determine seed thickness. Seed ranged in thickness from 1.4mm to 3.1mm. Germination was begun March, 1987, using 33 seeds from each size group in the range of 1.7mm to 2.5mm. Each millimeter increment was considered a group. The very small (1.4 to 1.6mm) and the very large (2.6 to 3.1mm) seed were not tested at this time due to the small number of seed in these groups.

Eleven seeds per 100x15mm petri dish were placed between two #1 Whatman filter papers and 9 ml water was added. Dishes were placed in a germination chamber at 32.5°C. After germination, seeds were transplanted to Speedling trays (Speedling, Inc.) in a greenhouse at the University of Arizona Campbell Agricultural Center, Tucson. In May, 113 plants were transplanted to the field at the Campus Agricultural Center.

During transplanting and subsequent cultivation in the field, some plants did not survive to maturity. Of the original planting of nine size groups (1.7mm to

2.5mm), 6 to 13 plants per size group were available for observation.

Pollination was allowed to occur naturally, and the plants were visited by a variety of insects, the primary pollinators being honeybees. When fruit had developed the plants were scored for ploidy level. Triploid fruit are light green with dark green stripes and tetraploid fruit are light green without stripes. By the end of July all the surviving plants had been scored.

The remaining three thickness groups (1.4 to 1.6mm, 2.6 to 2.8mm, and 2.9 to 3.1mm) were planted at this time in 20 liter pots in the greenhouse at the Campus Agricultural Center. Ten, 7 and 5 plants, respectively, of each of the above class thicknesses, were scored. Flowers were hand-pollinated using Calsweet diploid plants as pollinators. Fruit coloration was apparent when the fruits were about 3 inches in diameter. A t-test was used to determine whether there were any differences in seed thickness between triploid and tetraploid populations.

Evaluation of Seed Weight

The same seed lot obtained from Herb Partridge was separated into seed weight groups. Each seed was

individually weighed to the nearest 1.0mg on a Mettler balance. Seeds ranged in weight from 21mg to 110mg. Eighteen groups were formed, consisting of 5-milligram increments. Varying numbers of seed were germinated, starting with an optimal number of 30 seed per group. The smaller and larger weight groups had fewer seeds available for testing.

The seeds were placed on moist germination paper in plastic bags at 30°C for germination. As seeds germinated, they were planted into 19 l pots in the greenhouse at the Campus Agricultural Center, Tucson. Plants were hand-pollinated either by selfing or by using Calsweet diploids as pollinators. The plants could be scored for ploidy based on rind color within two weeks following fertilization. Plants were scored for about three months. As daylength shortened, flowering, especially pistillate flowering, declined to almost nothing.

Ploidy groups were again analyzed for differences in weight using a t-test. Seeds that were separated by size often had low germination percentages, leaving few plants to analyze. This led to the idea of using a pre-treatment technique to improve germination.

Improvement of Germination With Priming Solutions

The three osmotic solutions used for osmotic pre-treatment were distilled water, PEG 8000 (Sigma Chemical Co.) (-1.25mPa), and 3% KNO_3 (-1.25mPa). An untreated control was included in this study. Solutions were aerated using porous stone air releasers attached to plastic tubing and an aquarium air pump. Each treatment consisted of 60 seeds; 30 for germination testing and 30 for emergence testing. Treatments were begun in sequence, beginning at -13 days, so that all 19 treatments of one replication at one temperature would be placed into the germination chamber at the same time. The experiment was run at four temperatures: 30°C, 25°C, 20°C and 15°C, for 3 replications. Seeds were placed into the aerated solutions for 0,1, 3 or 6 d at ambient temperature ($\sim$24°C). They were then allowed to air-dry on paper toweling for 0, 1 or 7 d (table 1). The experiment was arranged as a split plot design, with each temperature representing the whole plot and treatments representing the subplots.

For germination, the dried seeds were dusted with Benlate and placed on moist germination paper, which was then folded in half and put into Zip-lock bags. The bags were left open to allow free circulation

TABLE 1. Factors and levels of factors for testing osmotic pre-treatment effects on germination and emergence under laboratory conditions.

Trttimes (days)	Drying times (days)	Temperatures (°C)	Osmotica
0	0	15	H_2O
1	1	20	PEG 8000
3	7	25	KNO_3
6		30	Untreated

of air, and were placed on one of two shelves of the germination chamber in a completely randomized design. The bottom of the germination chamber was filled with water to sustain a humid environment.

Aluminum pans filled with clean silica sand (#20) were used for the emergence test. Holes were punched in the bottom to allow for drainage. Before the seeds were planted the sand was thoroughly soaked and excess water allowed to drain off. The seeds were dusted with Benlate and placed approximately 2cm apart in three rows and barely covered with sand. The sand was approximately 5cm deep. Pans were placed in the germination chamber on four shelves in a completely randomized arrangement. Germination and emergence was counted at 7 d and 14 d. After the first count, sprouted seeds were removed. Each run was terminated after the second count. The positive results obtained in the laboratory led to the decision to direct-seed pre-treated seeds in the field.

Field Trial for Seed Priming

Seeds were treated for three lengths of time plus the control (0d, 1d, 3d, 6d), three osmotic solutions were used (H_2O, PEG, KNO_3), and all treatments were dried for

the same length of time (14d). The length of drying time was arbitrarily determined, as planting was scheduled to coincide with planting of other plots. Since analysis of variance for the lab experiments did not show significant differences for drying times, it was decided that this would not be a critical factor for the field trial. There were three replications, with 50 seeds in each replication. The field trial was analysed as a randomized complete block design.

The seeds from all three reps for an osmoticum were pre-treated together. After pre-treatment the seeds were rinsed in distilled water and laid on paper towels to dry at room temperature. When dry, the seeds were placed in marked envelopes by treatment and rep.

Seeds were planted into the field in a randomized complete block design. Emergence was counted once a week for the first four weeks after planting. Analysis of variance was used to determine statistical differences between treatments.

RESULTS

Germination of Tri x 313 Watermelon Seed

Germination percentages of the Tri x 313 seed are shown in Table 2 with mean separations. Mean germination ranged from 1.3± 1.1% to 6.8 ± 4.2% (Fig.1 and Table 2). This generally followed the pattern for seed size in that the larger seeds tended to germinate better than the smaller ones. These numbers were much too low for production standards and warranted discarding these seeds from further testing.

Separation of Seed by Thickness

In size groups that consisted of 33 seeds (1.7 to 2.5 mm), germination percentages ranged from 18% to 55%. In the smaller and larger size groups germination was 80% to 100% (Table 3, Fig.2). Separation of triploid and tetraploid seed based on thickness was not possible; there were no significant differences between populations.

Separation of Seed by Weight

One-hundred percent germination was not uncommon for this group of seeds (Table 4, Fig. 3). Overall, gemination tended to increase with increased seed size, but triploids did not seem to be either more or less

TABLE 2. Germination of Tri x 313 Seeds of five size groups, and the controls, Charleston Gray (CG) and Charleston Gray 5 (CG 5).

	Number of Seeds Germinated (per 100 seeds)						
	A*	B	C	D	E	CG	CG5
mean	1.25	1.75	1.5	4.25	6.75	80.5	93.75
std.dev.	±1.1	±1.3	±2.6	±2.9	±4.2	±5.5	±2.9
lsd	(d)	(cd)	(d)	(cd)	(c)	(b)	(a)

*)A= < 2.2mm
B= 2.2 to 2.4mm
C= 2.4 to 2.6mm
D= 2.6 to 2.8mm
E= > 2.8mm

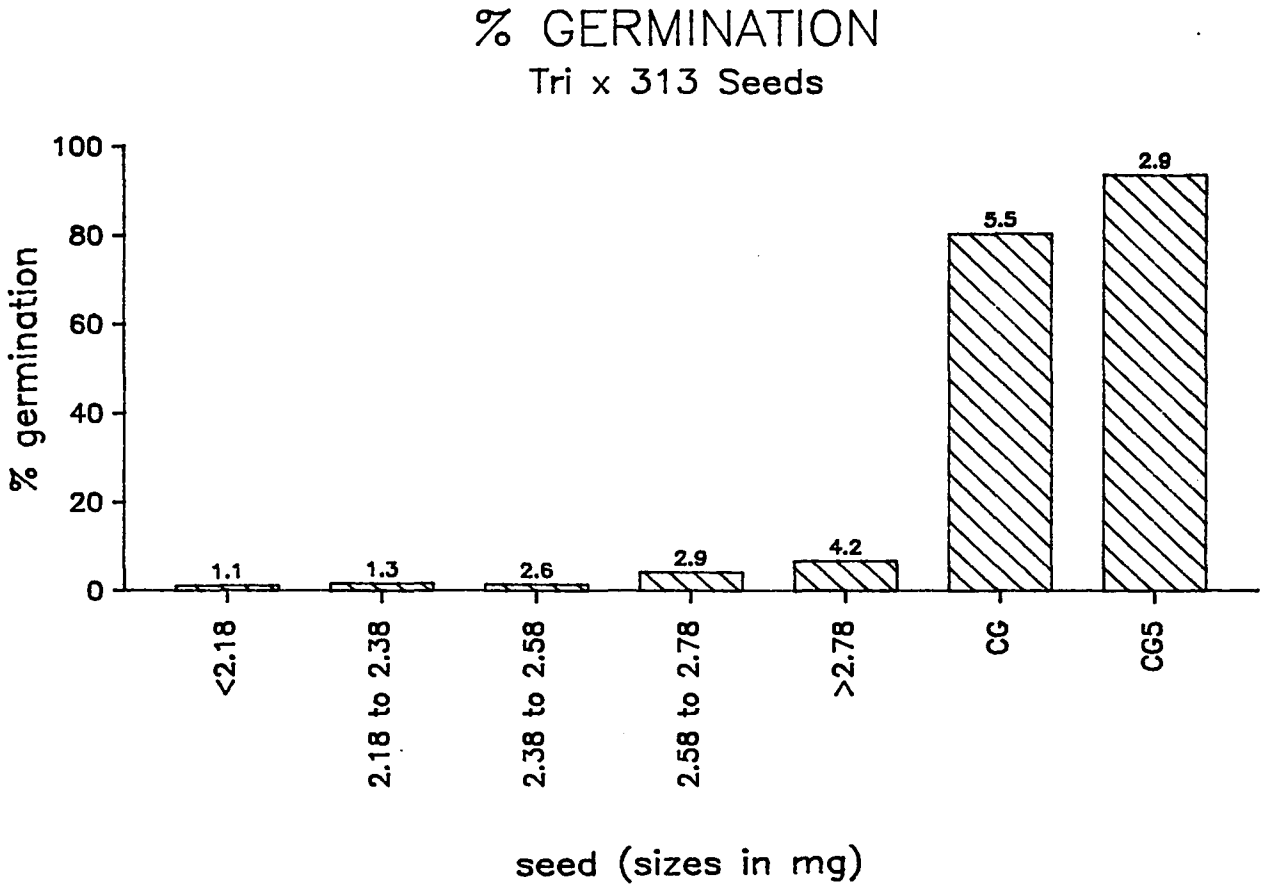

Figure 1. Germination of Tri x 313 seeds separated by thickness and of two diploid varieties, Charleston Gray and Charleston Gray No. 5.

TABLE 3. Germination percentages and ploidy of Jubilee seedless seeds separated by thickness.

Size (mm)	No. of seed	% Germ	No. scored 3N	No. scored 4N
1.4 to 1.6	15	80	4	6
1.7	33	55	4	7
1.8	33	42	8	3
1.9	33	39	4	5
2.0	33	27	4	9
2.1	33	30	4	7
2.2	33	18	1	5
2.3	33	21	4	5
2.4	33	30	3	4
2.5	33	30	5	5
2.6 to 2.8	15	80	5	2
2.9 to 3.0	6	100	1	4
Total			47	62
Mean			2.1±0.38	2.1 ±0.38[a]

a) t-value=0.00
$P_{0.05}$=1.98
P>0.90

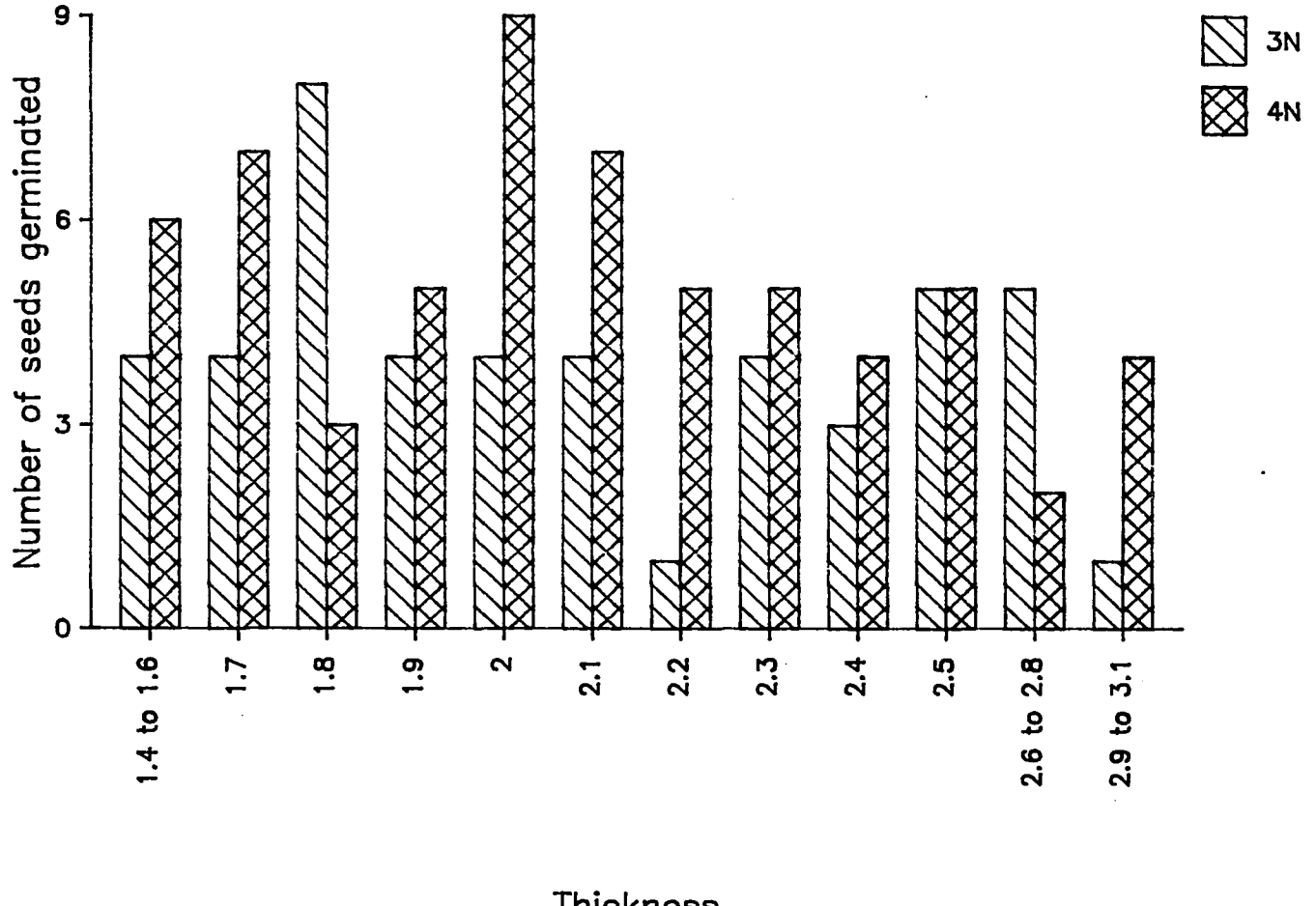

Figure 2. Germination and ploidy of Jubilee Seedless seeds separated by thickness.

TABLE 4. Germination percentages and ploidy of Jubilee Seedless seeds separated by weight.

Size (mg)	No. of seed	% Germ.	No. scored 3N	No. scored 4N
21 to 25	3	0	0	0
26 to 30	5	40	1	0
31 to 35	13	69	3	2
36 to 40	27	74	7	6
41 to 45	30	63	6	4
46 to 50	30	57	6	2
51 to 55	30	57	5	6
56 to 60	30	67	4	5
61 to 65	30	83	6	6
66 to 70	30	93	4	7
71 to 75	30	97	9	4
76 to 80	30	100	9	5
81 to 85	30	100	4	9
86 to 90	30	100	3	2
91 to 95	30	100	7	4
96 to 100	30	100	5	6
101 to 105	24	100	4	3
106 to 110	3	67	1	1
Total			84	72
Mean			67.88	69.52
Std. dev.			±21.39	±20.41[a]

a) t-value=0.50
$P_{0.05}$=1.96
P>0.90

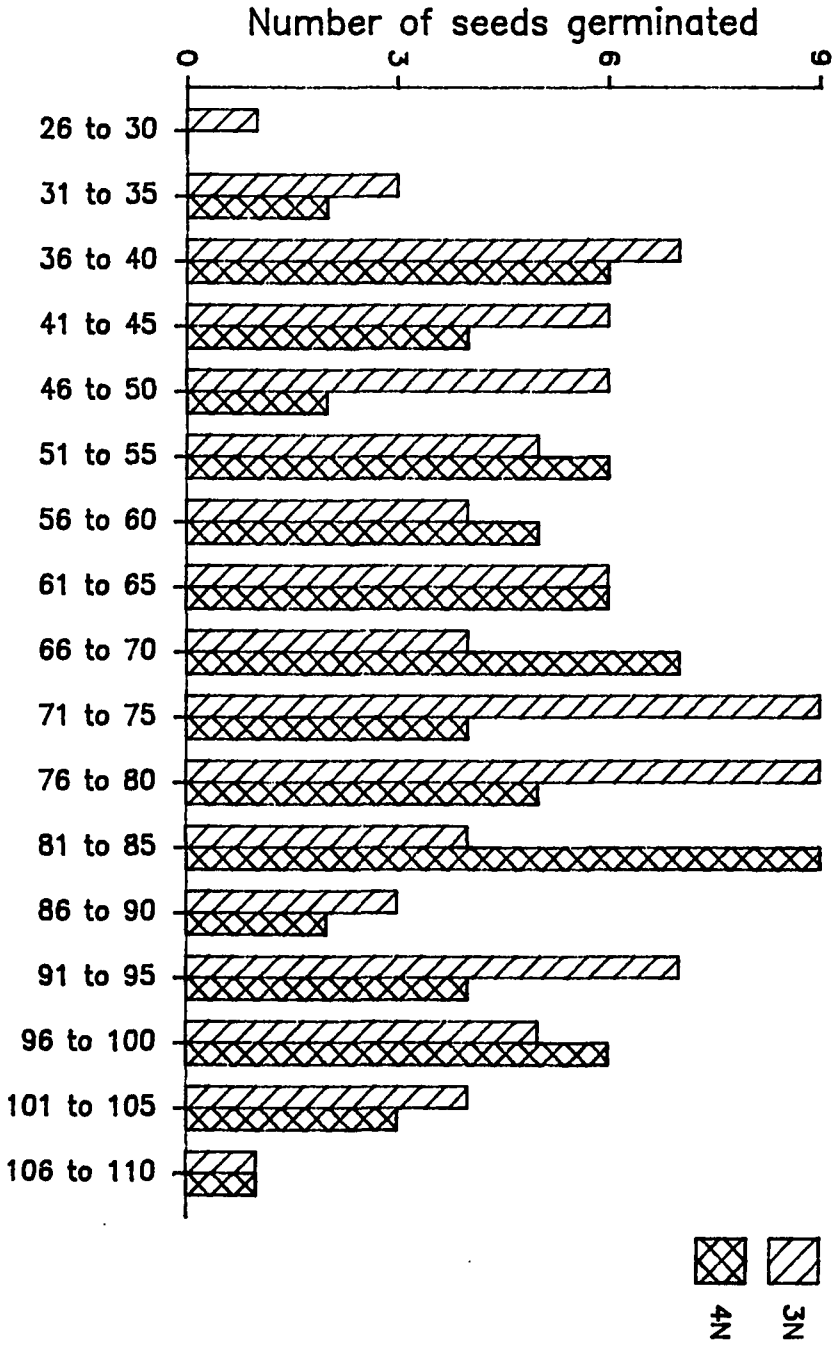

Figure 3. Germination and ploidy of Jubilee Seedless seeds separated by weight.

viable than tetraploids. Efforts to separate triploid and tetraploid seed were unsuccessful; the means and standard deviations were nearly equal.

Osmotic Pre-treatment and Laboratory Results

Table 5 gives the results of the analysis of variance (ANOVA) for the effects of pre-treatment on germination. The ANOVA for emergence in the laboratory trials is given in Table 6. Contrasts were calculated using the General Linear Model for differences between levels of a factor and for interactions between factors. Subplots were designated, combining all three factors (treatment time, drying time, and osmoticum), and separation of means using the Least Significant Differences (LSD) procedure was used to order the subplots. (Tables 7 and 8).

There were significant differences between levels of each variable, as well as interactive effects between variables. For both germination and emergence, one day in treatment improved germination more than 3d or 6d. There was no difference between drying for 1d or 7d on either germination or emergence. Pre-treatment in water for 1d improved germination over the control. This treatment was also a better osmoticum for improving

TABLE 5. Analysis of variance for osmotic pre-treatment effects on germination including contrasts analysed using the general linear models procedure.

Source		DF	SS	F Value	Prob.>F
REP		2	190	14.1	0.000 *
TEMP		3	648	31.9	0.000 *
REP*TEMP		6	372	9.2	0.000 *
SUBPLOT		18	3120	25.6	0.000 *
SUBPLOT*TEMP		54	485	1.3	0.095
CONTRASTS(a)					
Trt time:	1d vs 3d	1	355	52.4	0.000 *
	1d vs 6d	1	198	29.3	0.000 *
	3d vs 6d	1	13	1.9	0.16
Dry time	1d vs 7d	1	6	0.89	0.000 *
Osmo.	H_2O vs PEG	1	2475	365.6	0.000 *
	H_2O vs KNO_3	1	702	103.7	0.000 *
	PEG vs KNO_3	1	2352	0.03	0.000 *
Interactions					
	Trt vs Dry	2	46	3.4	
	Trt vs Osmo	4	96.4	3.6	0.008 *
	Dry vs Osmo	2	81	5.9	0.003 *
	Trt/Dry/Osmo	4	65.9	2.4	0.050 *
ERROR		144	974	6.8	
TOTAL		227	5791		

*denotes significance at the 0.05 level

(a) Contrasts were done separately and were included in this table for ease of reference. Therefore, the degrees of freedom for them does not add up to 18, nor are they added into the total DF of 227.

TABLE 6. Analysis of variance for effects of osmotic pre-treatment on emergence.

Source		DF	SS	F Value	Prob.>F
REP		2	428	37.8	0.00 *
TEMP		3	467	27.46	0.00 *
REP*TEMP		6	187	5.49	0.00 *
SUBPLOT		18	903	8.85	0.00 *
SUBPLOT*TEMP		54	440	1.44	0.46 *
CONTRASTS[a]					
Trt time:	1d vs 3d	1	312	55	0.00 *
	1d vs 6d	1	152	26.8	0.00 *
	3d vs 6d	1	18	3.2	0.07
Dry time	1d vs 7d	1	0.7	0.12	0.73
Osmo.	H_2O vs PEG	1	572	100.9	0.00 *
	H_2O vs KNO_3	1	81	14.3	0.00 *
	PEG vs KNO_3	1	992	175	0.00 *
Interactions					
	Trt vs Dry	2	0.3	0.03	0.99
	Trt vs Osmo	4	41	1.81	0.50
	Dry vs Osmo	2	1.8	0.16	0.95
	Trt/Dry/Osmo	4	18	0.78	0.95
ERROR		144	816	5.7	
TOTAL		227	3241		

*denotes significance at the 0.05 level

(a) Contrasts were done separately and were included in this table for ease of reference. Therefore, the degrees of freedom for them does not add up to 18, nor are they added into the total DF of 227.

TABLE 7. Mean separation by the least significant differences test for osmotic pre-treatment effects on germination.

T-grouping	Osmo.	Trt.(d)	Dry Time(d)	Mean Germ.
A	H_2O	1	1	13.7
A	H_2O	3	1	13
A	H_2O	1	7	12.9
B	Control	0	0	10
BC	H_2O	6	7	8.9
BC	H_2O	6	1	8.8
CD	H_2O	3	7	7.7
CD	KNO_3	1	7	7.7
D	KNO_3	6	7	6.6
D	KNO_3	3	7	6.4
D	KNO_3	3	1	6.1
DE	KNO_3	6	1	5.8
DE	KNO_3	1	1	5.8
EF	PEG	1	7	3.9
FG	PEG	1	1	3.7
FG	PEG	3	7	2.1
FG	PEG	3	1	2.0
FG	PEG	6	1	1.9
G	PEG	6	7	1.6

TABLE 8. Mean separation by the least significant differences test for osmotic pre-treatment effects on emergence.

T-grouping	Osmo.	Trt.(d)	Dry Time(d)	Mean Germ.
A	H_2O	1	7	8.3
AB	Control	0	0	7.0
AB	H_2O	1	1	6.9
ABC	H_2O	3	1	6.5
BCD	H_2O	3	7	6.0
BCDE	KNO_3	1	7	5.4
CDE F	KNO_3	1	1	4.7
CDEF	KNO_3	3	1	4.7
CDEF	KNO_3	6	7	4.6
CDEF	H_2O	6	1	4.6
DEF	H_2O	6	7	4.3
DEF	KNO_3	3	7	4.3
DEF	PEG	1	1	4.2
FG	KNO_3	6	1	3.8
FG	PEG	1	7	2.9
G	PEG	3	7	1.8
G	PEG	6	1	1.5
G	PEG	6	7	1.3
G	PEG	3	1	1.0

germination or emergence than treatment in KNO_3, which, in turn, was more successful than PEG.

In Table 9 the germination results are presented by osmoticum, treatment time and temperature, with percentages given in parentheses. Figures 4-7 present the information from this table for each temperature. Pre-treatment in H_2O allowed higher germination than PEG or KNO_3. The separation of means shows that, while all treatments in the H_2O did not improve germination over untreated seeds, 1d in pre-treatment was better than no treatment.

Figure 8 presents the interactive effects of osmoticum and temperature, with maximum germination ($\bar{x}$=43% in H_2O) at 20°C. The control seeds improved at each increase in temperature, with maximum rates achieved at 30°C.

Figure 9 shows the interaction of osmoticum, days in treatment, and drying time. With H_2O, one day pre-treatment followed by one day drying produced the most beneficial results (45.6%), while three days in pre-treatment followed by 7 days drying produced the least beneficial results (27.2%). The lowest results overall were achieved with PEG; germination was below 15% regardless of the length of treatment.

TABLE 9. Effects of osmotic pre-treatment on germination under laboratory conditions.

		No. of seeds germinated (%)			
OSMO	TRT TIME	T=15°C	T=20°C	T=25°	T=30°
H_2O	6d	43 (24)	58 (32)	53 (29)	58 (32)
H_2O	3d	38 (21)	75 (42)	61 (34)	74 (41)
H_2O	1d	59 (23)	99 (55)	91 (51)	70 (39)
PEG	6d	3 (2)	15 (8)	9 (5)	12 (7)
PEG	3d	11 (6)	10 (5)	17 (9)	11 (6)
PEG	1d	9 (5)	27 (15)	32 (18)	23 (13)
KNO_3	6d	13 (7)	48 (27)	45 (25)	43 (24)
KNO_3	3d	6 (3)	47 (26)	47 (26)	50 (28)
KNO_3	1d	13 (7)	49 (27)	51 (28)	49 (27)
Control	(None)	25 (28)	27 (30)	29 (32)	39 (43)
Mean Separation		b	a	a	a

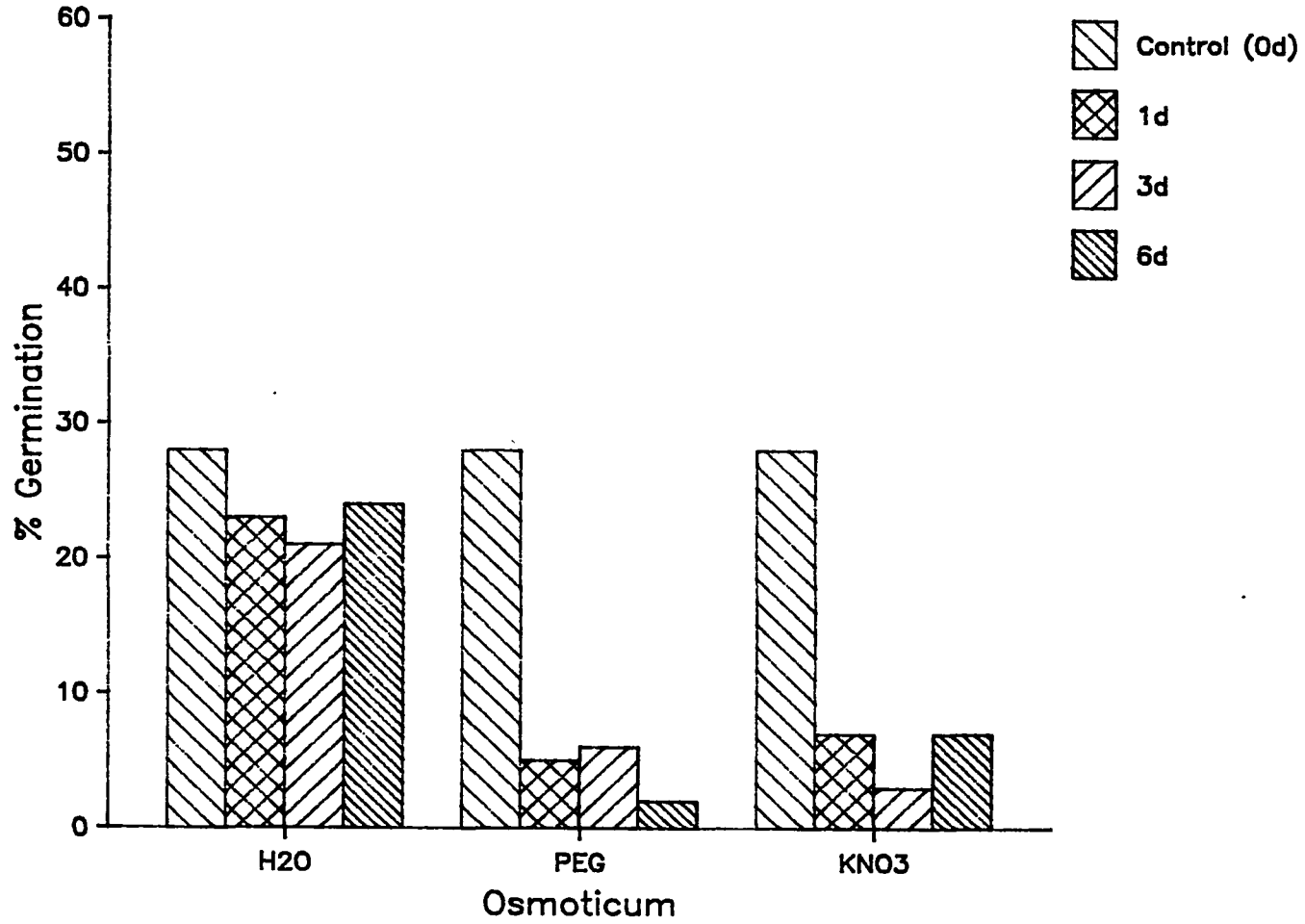

Figure 4. Percent germination at 15°C of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

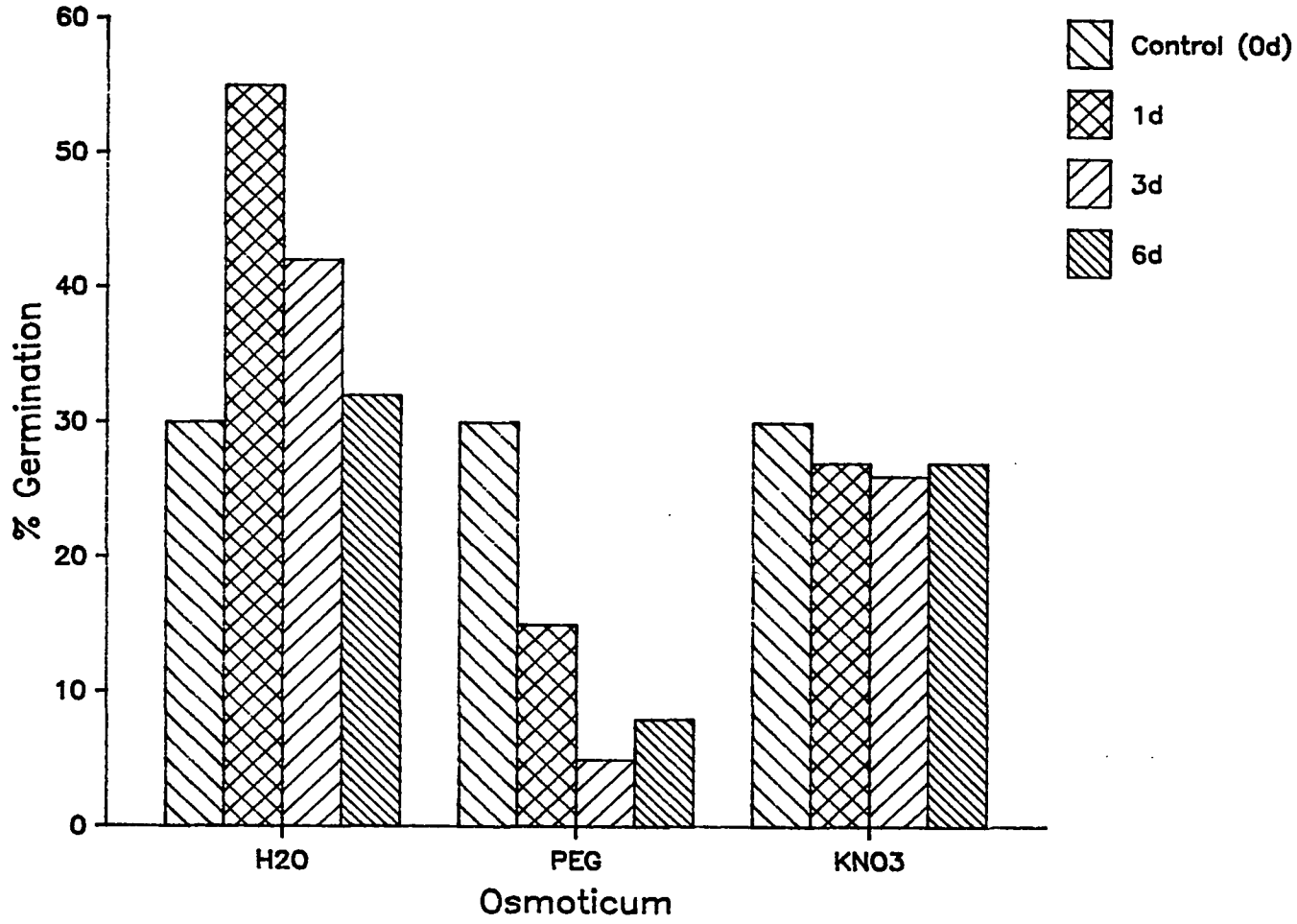

Figure 5. Percent germination at 20°C of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

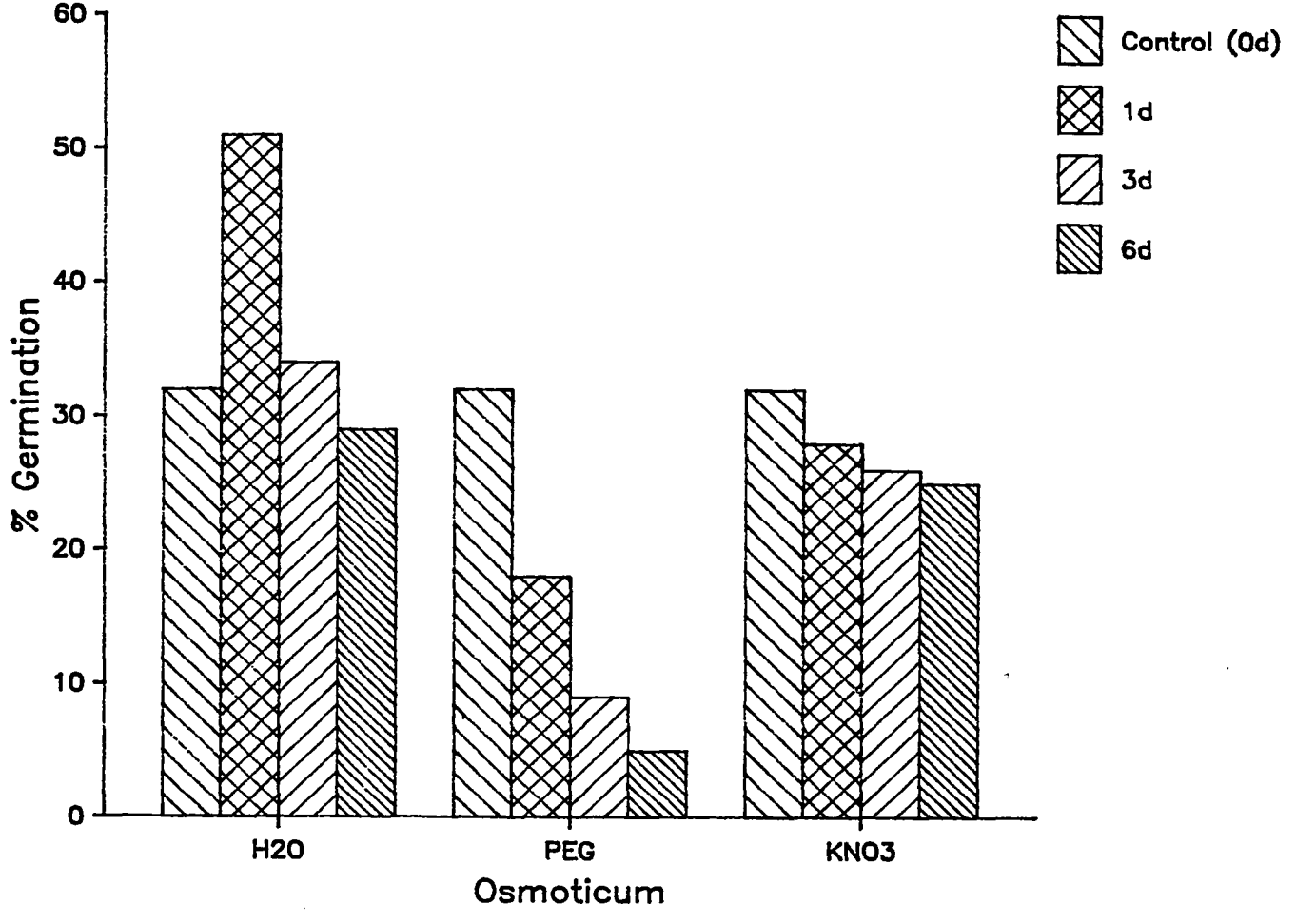

Figure 6. Percent germination at 25°C of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

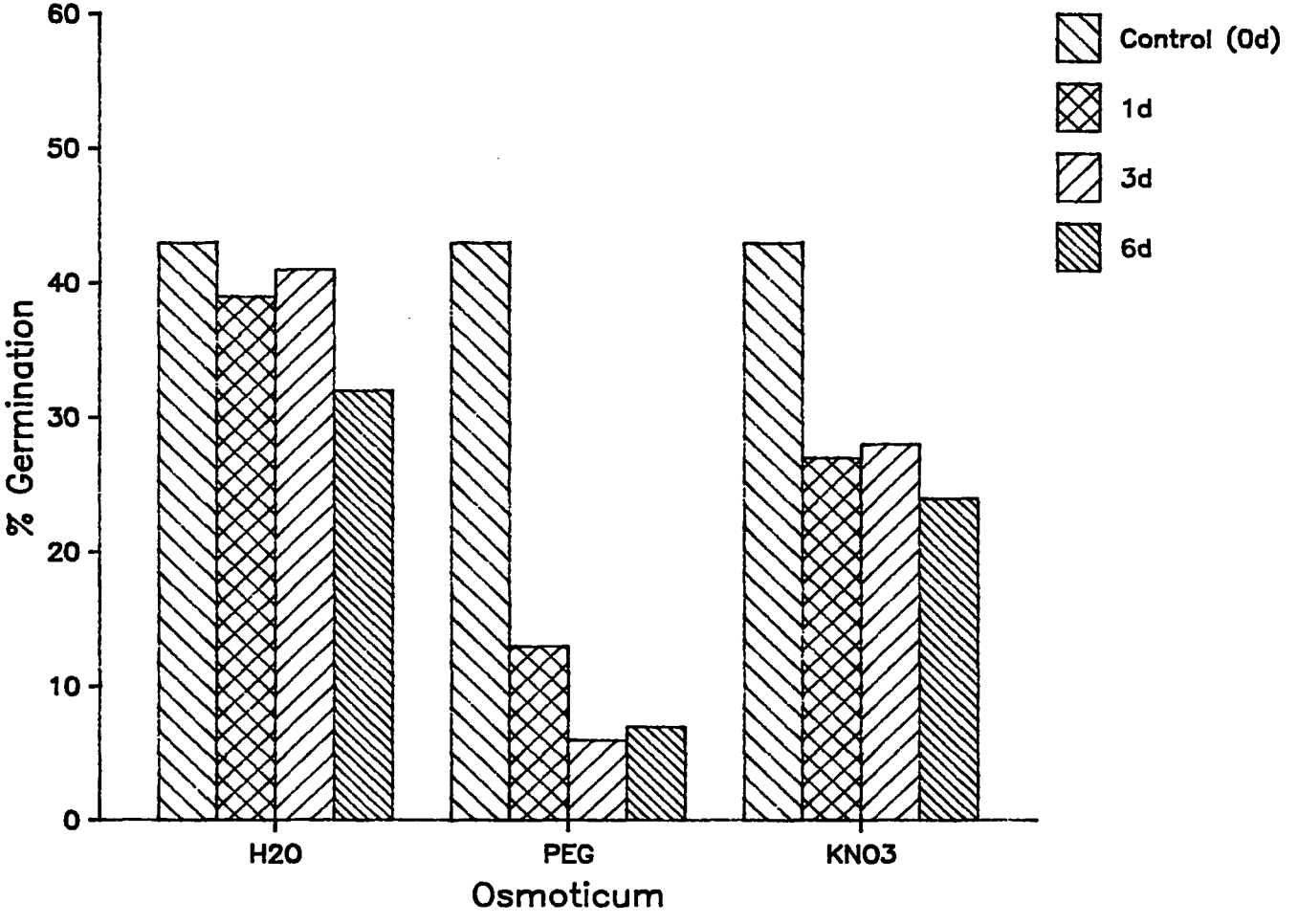

Figure 7. Percent germination at 30°C of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

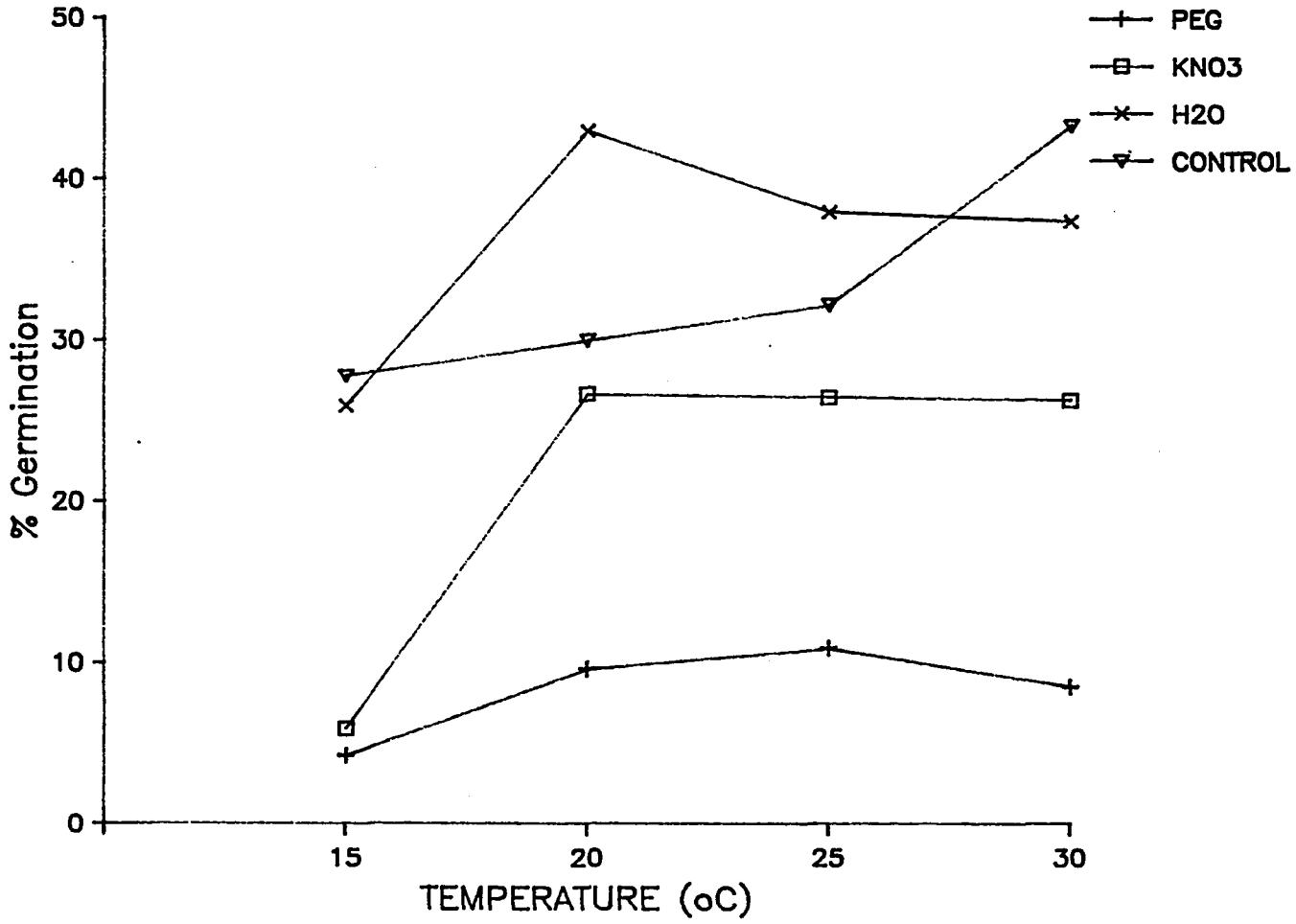

Figure 8. Interaction of osmoticum and temperature on germination of pre-treated seeds.

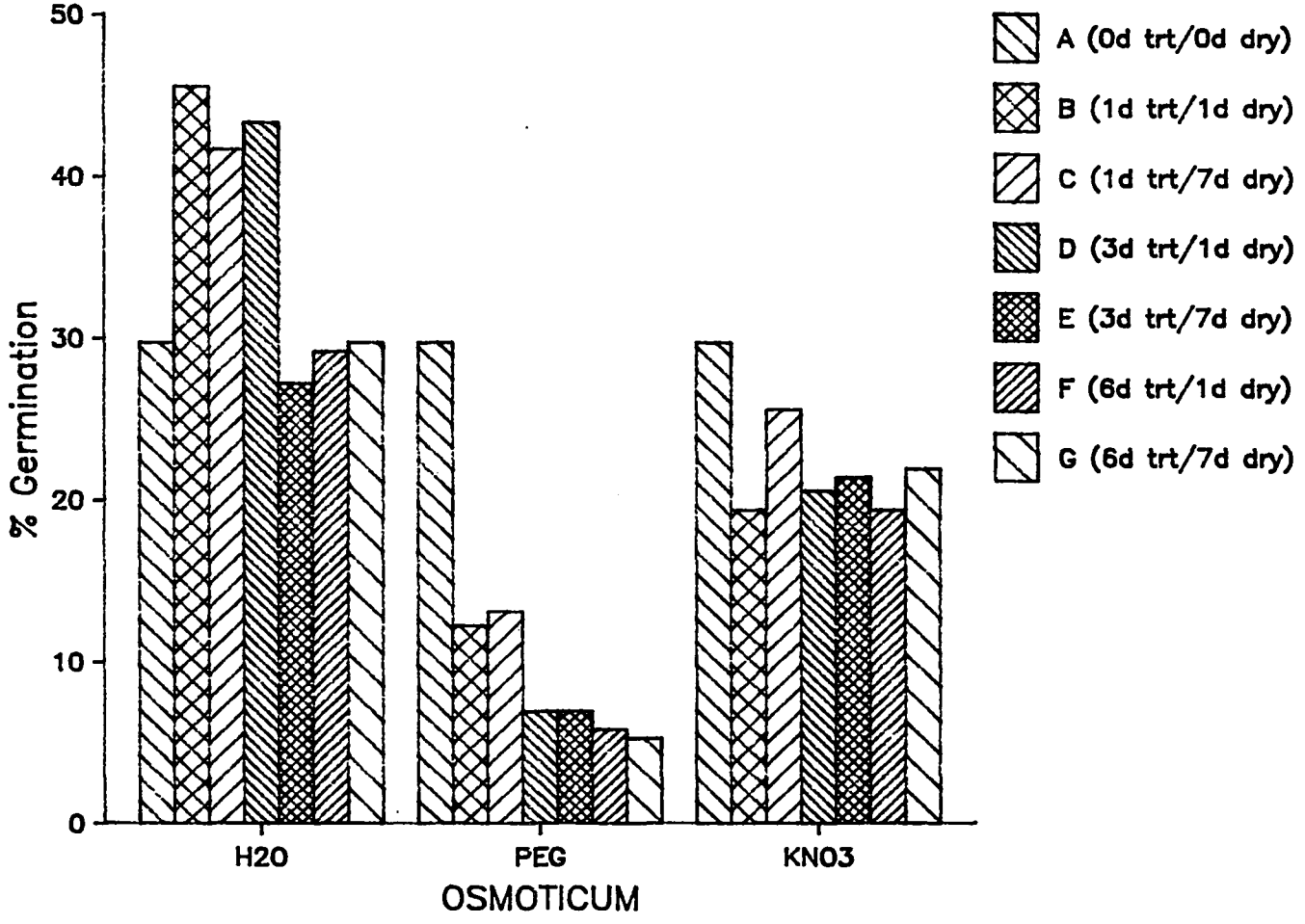

Figure 9. Interaction of osmoticum, length of treatment, and length of drying time on germination of pre-treated seeds.

In Table 10 the emergence results are arranged by osmoticum, treatment time and temperature. Pre-treatment in PEG or KNO_3 did not improve germination over the control. At 25°C, pre-treatment for 1d in H_2O improved germination (38%) over the control (28%) (Figs. 10-13).

Figure 14 presents the interactive effects on emergence. Again, there were interactions between osmoticum and temperature (Fig. 14). Pre-treated seeds had maximum emergence rates at 25°C, with H_2O the highest at 29.3%. The control seeds had a maximum germination of 28.9% at 20°C. At the extreme temperatures the osmotica seemed to work against germination.

Osmotic Pre-Treatment and Field-Emergence

Analysis of variance did not render any significant differences between treatments, due either to osmotica or number of days in treatment (Table 11). Germination percentages of seeds that were planted in the field are given in Table 12 and Figure 15. All the pre-treated seeds exhibited better germination than the control, but none were statistically significant. In general, the germination percentages were unacceptably low.

TABLE 10. Effects of osmotic pre-treatment on emergence under laboratory conditions.

		Number of seeds emerged (%)			
OSMO	TRT TIME	T=15°	T=20°	T=25°	T=30°
H_2O	6d	16 (9)	33 (18)	31 (17)	27 (15)
H_2O	3d	19 (10)	40 (22)	51 (28)	40 (22)
H_2O	1d	19 (11)	61 (34)	68 (38)	29 (16)
PEG	6d	9 (5)	10 (5)	9 (5)	5 (2)
PEG	3d	8 (4)	10 (5)	14 (8)	2 (1)
PEG	1d	10 (5)	24 (13)	40 (22)	11 (6)
KNO_3	6d	20 (11)	33 (18)	31 (17)	17 (9)
KNO_3	3d	17 (9)	16 (9)	52 (29)	23 (13)
KNO_3	1d	22 (12)	28 (16)	47 (26)	25 (14)
Control	(None)	11 (12)	26 (29)	25 (28)	22 (24)
Separation of means		d	c	b	a

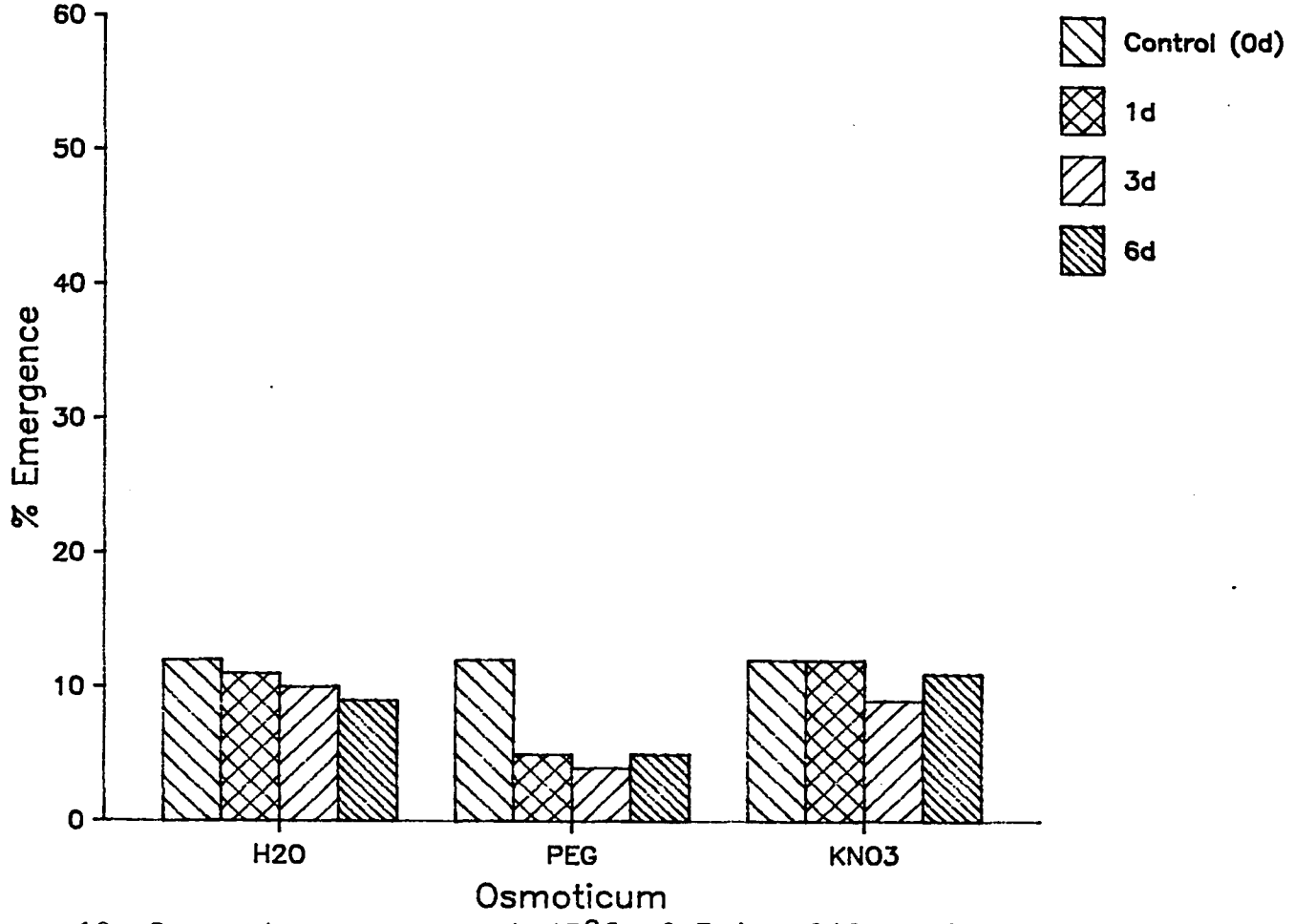

Figure 10. Percent emergence at 15°C of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

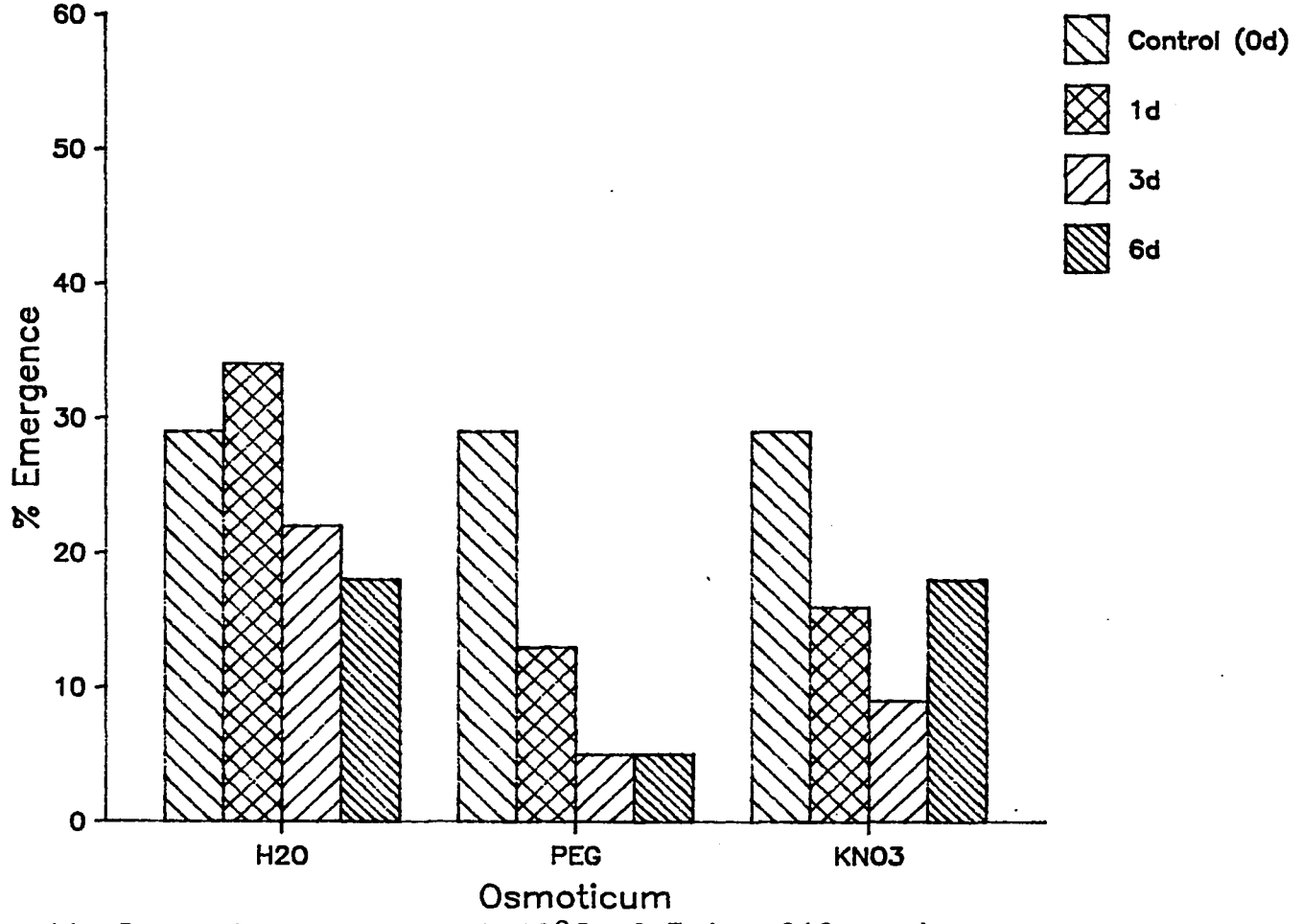

Figure 11. Percent emergence at 20°C of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

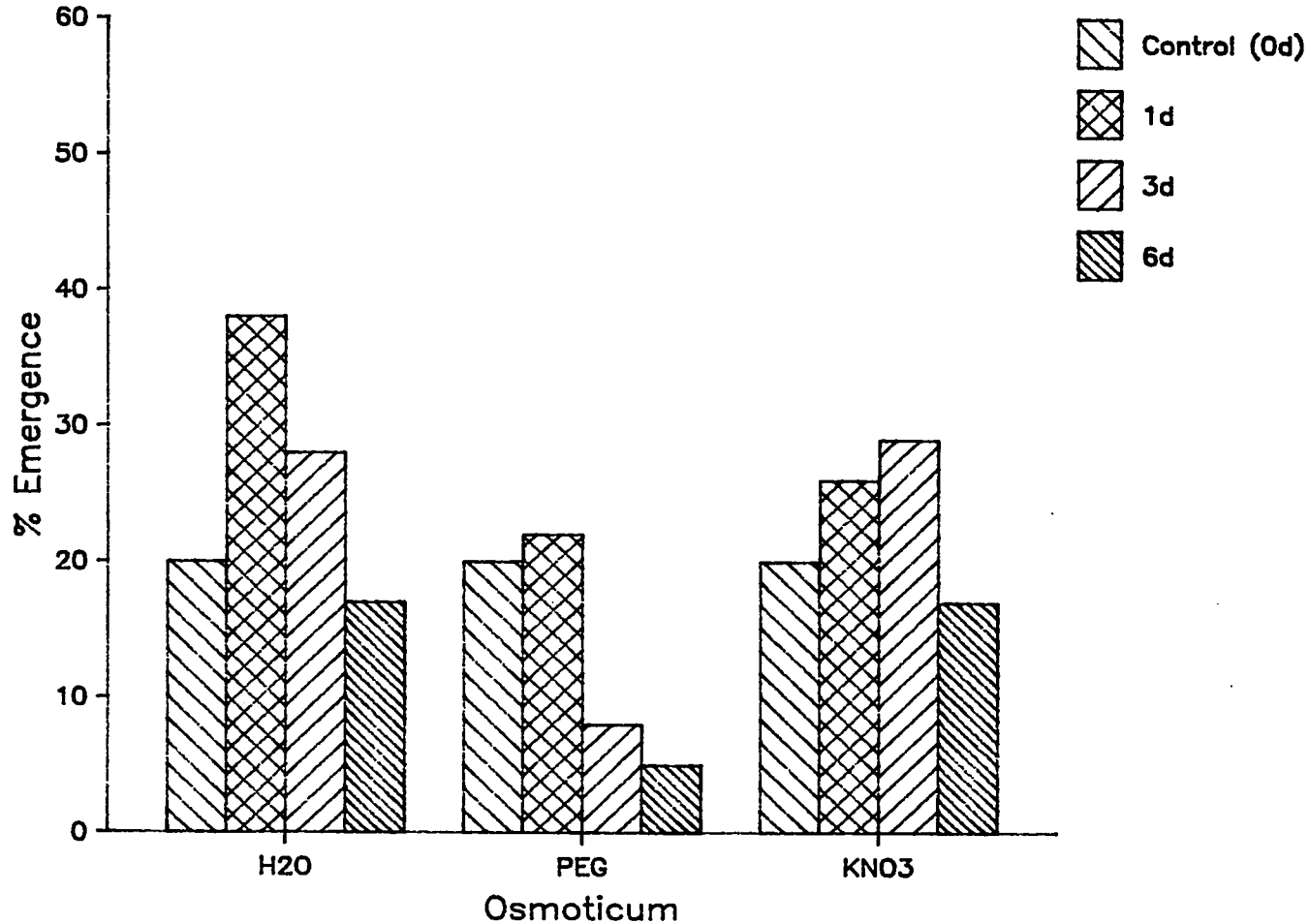

Figure 12. Percent germination at 25°C of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

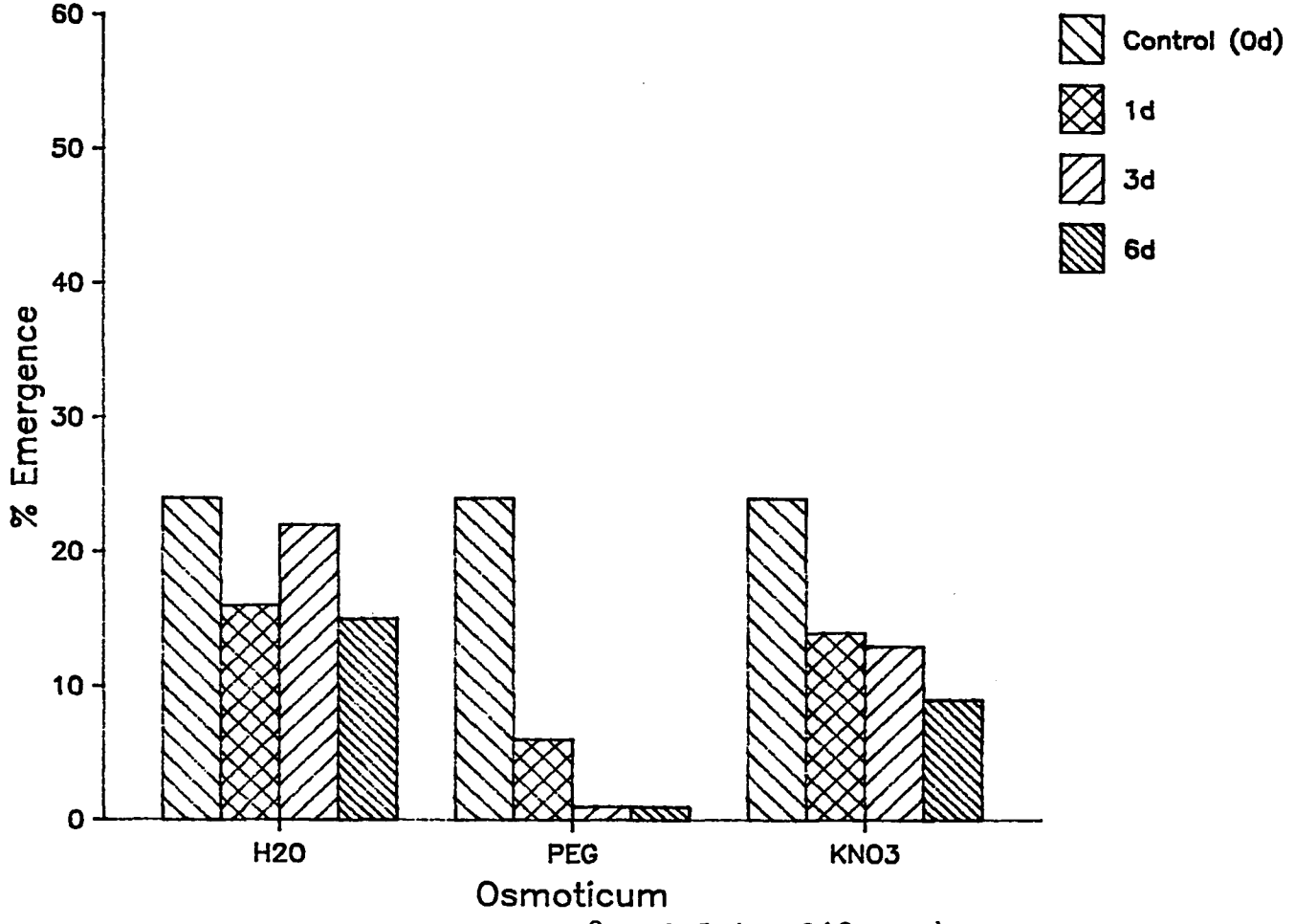

Figure 13. Percent germination at 30°C of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

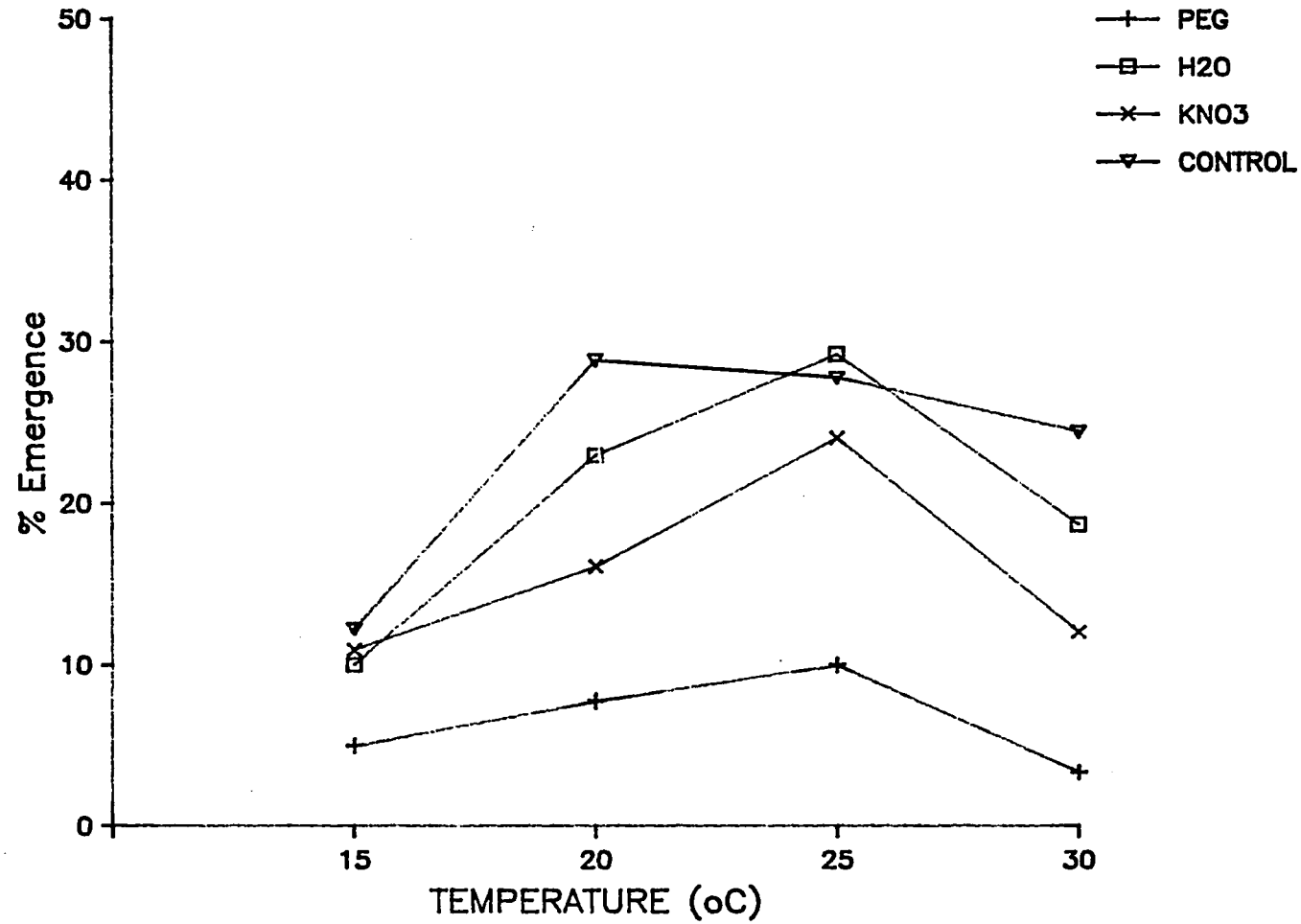

Figure 14. Interaction of osmoticum and temperature on emergence of pre-treated seeds.

TABLE 11. Analysis of variance to test osmotic pre-treatment effects on field emergence.

Source	DF	Sum of Squares	Mean Square	F Value	Prob.>F
TRT TIME	3	9.91	3.30	0.36	0.7855
OSMOTICUM	3	13.47	4.49	0.48	0.6979
TRTxOSMO	3	0.09	0.03	0.01	0.9997
REP	2	4.87	2.43	0.26	0.7723

*denotes significance at the 0.05 level

TABLE 12. Effects of osmotic pre-treatment of watermelon seeds on emergence under field conditions.

OSMO	TRT TIME	No. Emerged (%)
H_2O	6d	19 (12.67)
H_2O	3d	22 (14.67)
H_2O	1d	18 (12)
PEG	6d	20 (13.33)
PEG	3d	15 (10)
PEG	1d	14 (9.33)
KNO_3	6d	18 (12)
KNO_3	3d	18 (12)
KNO_3	1d	19 (12.67)
Control	(None)	13 (8.67)

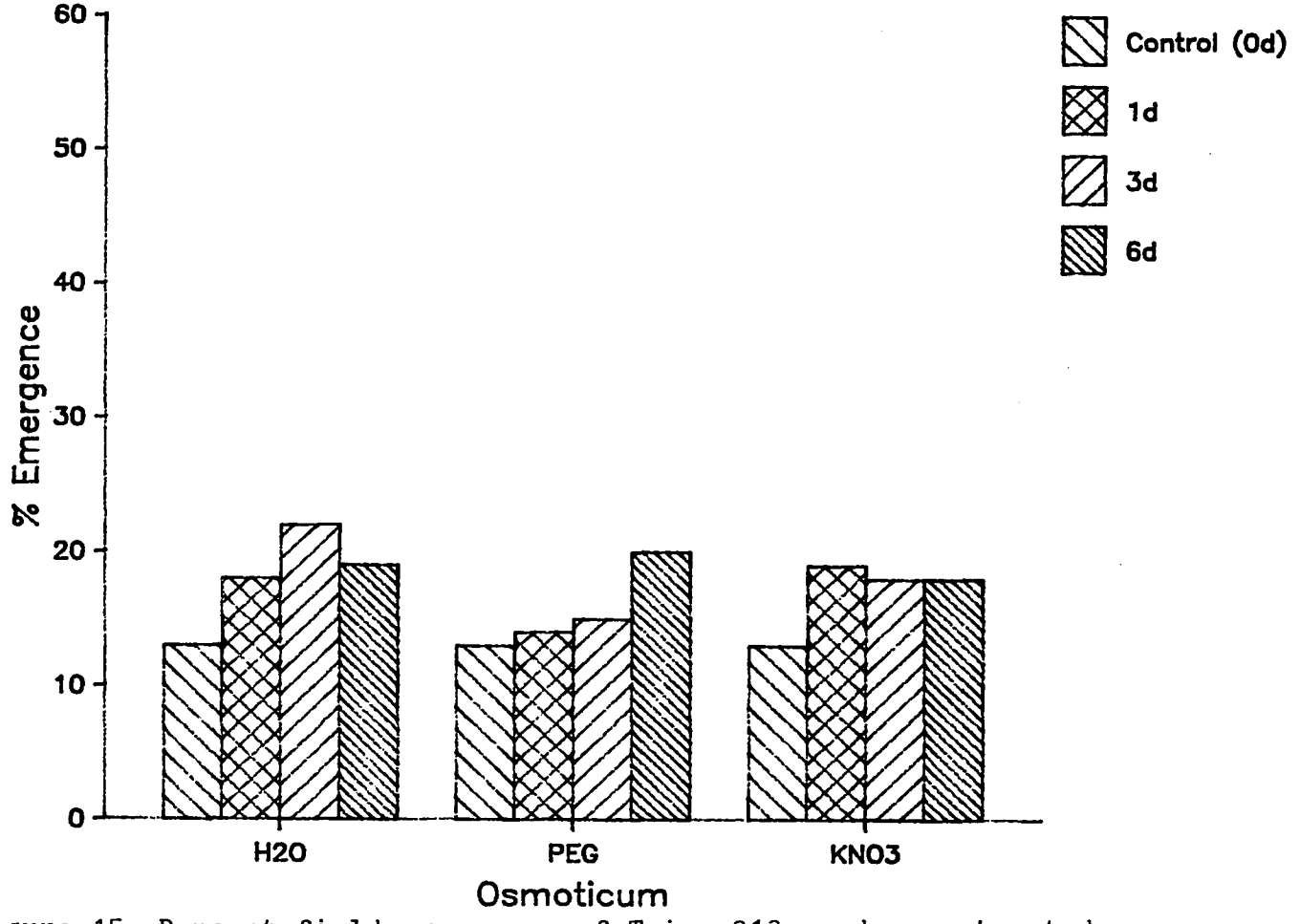

Figure 15. Percent field emergence of Tri x 313 seeds pre-treated in osmotic solutions for varying lengths of time.

DISCUSSION

The slower germination of the Tri x 313 seed resulted in a longer imbibition time, which may have adversely affected some embryos by causing them to become waterlogged. This would help explain their reduced germinability. In contrast, the diploid varieties, which germinated after only 2 or 3 days, quickly used up the moisture in the petri dishes.

While the same germination technique (using petri dishes) was used in germinating the Jubilee Seedless seeds that had been separated by thickness, a new method (using germination paper) was developed subsequently. The superior quality of the new method became evident in the high germination achieved in the final germination trial of the Jubilee Seedless seeds. However, germination rates in the field trial, even of the untreated seeds, were low again. These Tri x 313 were from a different seedlot than those used in the early part of this experiment. But the method used was the newer one. The highest percentages reached were 57% germination at 30°C and 43% emergence at 21°C. This can most simply be explained by differences in seedlot or differences in the environment that were not measured or observed. One other possibility is that the Jubilee

Seedless seeds are superior in viability to the Tri x 313 seeds. It is difficult to deduce this without running a trial of both together, under the same conditions.

Separation of Seed by Size

Malek-Hedayat (1981) found significant differences between seed weight of Gossypium hirsutum L. aneuploid (monosomic) and disomic seed. But the ability to separate watermelon seeds of different ploidy levels (let alone of differing chromosome numbers) does not appear to be possible.

Efforts to separate triploid and tetraploid seeds by size were not successful in this experiment. This may be due to the years-long commercial practice of direct-seeding tetraploids. Over time, more and more tetraploid seed were selected for earliness in germination, emergence, and early fruit-production. This inadvertant selection would have resulted in thinner seed coats and highly viable embryos. If there were at one time two populations (Shimotsuma and Matsumoto, 1957), such selection may have reduced their differences. In light of the information presented, it may be more beneficial to look for distinguishing seedling characteristics. If such characteristics were

found prior to transplanting, elimination of the unwanted plants might still be economical.

Priming Effects on Germination and Emergence

The complexity of this experiment tended to detract from the overall results obtained. Even with pre-treatment, germination percentages tended to remain low. The field trial exhibited this problem clearly. In one plot only 1 seed of the 50 planted, emerged. Even in the lab, emergence was often lower than germination in the final count. The problem seems to lie as much in the thick seed coat as in any physiological or genetic factor involved.

Water as a pre-treatment produced better results than PEG or KNO_3. This coincides with the account of 'chitting' given by Heydecker et al.,(1973). This is an age-old custom of beginning germination and bringing seeds to the point of radicle emergence. These seeds must then be kept moist until they are hand-sown or fluid-drilled. The disadvantages of this method are that the seeds must be kept moist, and, if the radicles actually emerge, care must be taken to prevent their breakage. The use of salt solutions are beneficial because they extend the 'chitting' time by creating a negative water potential. So the seeds may imbibe water

and begin the physiological processes of germination, with decreased radicle emergence. They are then removed from the solution, and planted at a later date.

Polyehtylene glycol and KNO_3 apparently slowed germination, or else they actually disrupted the physiological processes required for germination. When watermelon seeds are provided with optimal conditions for germination, they usually do so in 2 to 7 days. In this experiment, after pre-treatment, the seeds were left in the growth chamber for 14 days. After this time, all of the viable seeds should have germinated. Perhaps, though, the seeds that had been in PEG or KNO_3 had not yet overcome the inhibiting effects caused by these chemicals. This does not coincide with results such as those reported by Khan, et al. (1978). They used only PEG as an osmoticum and compared treated vs. untreated pea seeds. They measured increased synthesis of RNA; increased synthesis and rate of proteins; increased quality of soluble proteins; and increased esterase and acid phosphatase activity. Even though they did not compare seeds treated in PEG with those treated in water, there was an improvement over untreated seeds, unlike the present study.

Perhaps the important effect, in the case of watermelons, involves the seed coat, rather than the embryo. Soaking in a wet medium serves to soften the seed coat, which in the case of polyploids, is thicker than normal varieties. Water would serve this purpose more efficiently and quickly.

An observation made during the experiments was that, even though all treatments were dusted with Benlate, the control (no pre-treatment) always had more fungal growth than any of the other treatments. Some treatments experienced very little fungal problems, and these were often isolated around a single, non-germinating seed. The pre-soaking apparently served to rinse spores off the seed coat (and out of the fissures), and, possibly to create an adverse environment for their survival.

The field trial of pre-treated seeds was dismal. Germination as low as 0.5% for a control group ranging up to only 24% for pre-treatment in water cannot be considered acceptable for commercial practice. This reflects the results from the laboratory studies, where germination was nearly always higher than emergence under the same environmental conditions. These results indicate the trouble always experienced with seedless

watermelon production: poor germination of polyploid seed.

Future Research Objectives for Production of Seedless Watermelons

Solutions to production problems may not lie in seed separation techniques or in priming, but in an alternate way to produce seedless watermelon. Three possibilities come to mind: 1) chemical manipulation to stimulate parthenocarpy; 2) chemical manipulation to reduce staminate flowering in the 4N female, thus reducing self-pollination; and 3) discovery or induction of a mutation for male sterility that does not affect female sterility, for use as the female parent.

Wong (1941) found that parthenocarpic fruits could be induced using various hormones including naphthalenacetic acid (n.a.), its potassium salt (k.n.a.), 1% n.a. plus 0.5% colchicine, and 1% indolebutyric acid (IBA) plus 1% n.a. But the resulting fruit were often only partially developed, tended to be smaller on average than pollinated fruit with seeds and had thicker rinds. Colchicine-treated fruit were firm, but not as juicy as normally-developed fruit. Application of hormones to a cut style caused blossom-end scar tissue to form.

These problems do not bode well for production of seedless watermelons by chemical manipulation, but other growth regulators have been isolated which could be tested. The drawbacks include: 1) labor-intensity if chemicals have to be applied manually; 2) adverse environmental effects if the chemicals are flown on; and 3) cost-effectiveness. The latter could be improved if the fruit are indeed seedless and are of superior quality. This method would eliminate the need to create polyploidy. Indeed, already-improved diploid varieties could be used.

An early theory discussed by Whitaker (1931), is that sex determination in cucurbits takes place before meiosis. Omini and Hossain (1987) suggest that endogenous levels of some hormones control sex expression in the undeveloped buds. Whitaker reports on a study done by Heyer in 1884 wherein staminate to pistillate flower ratio could be increased by growing cucumbers in sand rather than in the greenhouse. Presumably some potting soil mix was used in the latter, but fertility of the soil was not given. Omini and Hossain (1987) studied the effects of fertilizers on Luffa cylindrica L. Roem. In general, potassium-containing fertilizers encouraged staminate flowering

while reducing pistillate flowering. Nitrogen and phosphorous fertilizers increased pistillate flowering and reduced staminate flowering. But they reject the notion that fertilizers have a direct effect on sex expression, attributing the effects instead, to hormone levels. They suggest that levels of certain hormones are affected by specific nutrients, but offer no evidence of this. Plants that favor pistillate expression have been found to have higher endogenous levels of auxin (Galun, et al., 1965; Heslop-Harrison, 1956).

The picture is not very clear when explanations of control over sex-expression turn to hormones. Increasing pistillate flowering is not enough to eliminate self-polination if staminate flowers still outnumber pistillate flowers 5 to 1 (Omini and Hossain, 1987). Yet, to really understand the problem, and thus to offer a solution, is going to require quite a bit more study. The modus operandi of auxins, cytokinins, ethylene and gibberellic acid are complex and interconnected in ways not well understood. We can conclude that application of auxins to decrease the staminate/pistillate flower ratio would be interesting to study, but prohibitively expensive at the production level.

The use of male-sterility is probably the best long-term solution for production of seedless watermelons. A male-sterile mutant has been created by use of irradation (Watts, 1962), and the gene that controls this appears to be closely linked to a gene that controls glabrousness. These male-sterile-glabrous (gms) plants exhibit reduced female-fertility, progeny of selfed plants suffer reduction in vigor, and their fruit is often of a lower quality (Watts, 1962). Love et al. (1985) successfully attempted to introduce this gms trait into tetraploid lines. If this gene were simply inherited, it could be quite useful in hybrid triploid production. But Ray and Sherman (1988) showed that male sterility in the gms mutant was due to desynapsis of homologous chromosomes, thus explaining the lower fertility.

Irradiation techniques can and should be employed in an effort to induce other types of male-sterility such as reciprocal translocations. Shimotsuma (1968) has made substantial progress in this area, achieving various ring-configurations at metaphase I, with pollen fertility as low as 6%. Ming, et al., (1988) also induced abnormal chromosome arrangements using gamma irradiation. They were able to reduce the rate of

seed set to 50 to 80% of that of the diploid varieties. They also found a few plants that were extremely female fertile. These set fruit at nearly every node, and all the fruits grew to maturity. The translocations strains used by Ming, et al. (1988) were also successfully crossed with the commercial diploid varieties Charleston Gray and Crimson Sweet. Fruit were obtained and seed set was reduced to as low as 25 seeds per fruit.

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