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PRE-SOWING TREATMENTS FOR BREAKING SEED DORMANCY IN RELATION TO GERMINATION, SEEDLING VIGOUR AND YIELD OF TEOSINTE

Aboelgoud, Sh.A.
Forage Crops Research Department, FCRI, ARC, Giza,Egypt

ABSTRACT

Laboratory and field experiments were conducted at the Laboratory of Seed Technol. Research Unit, Mansoura and El-Serw Experimental Farm Station Damietta Governorate, during the seasons of 2014 and 2015.

The variation in percentage germination of seed teosinte was found to be a function of dormancy breaking treatments even in genetically uniform seed lots. The aim of the present investigation was to determine the effect of pre-sowing treatments on breaking dormancy of the three colour degrees), of teosinte seed i.e. (white, cream and mixed between them) and subsequent improving its germination, seedling vigor, field emergence and crop yield. The results revealed that Seed germination and seedling vigor traits were found to be related to seed colour where seeds of cream colour showed high germination and produced vigorous seedling as compared with white and mixture of the two colours. Manual scarification improved seed germination and seedling vigor traits, while GA_3, sulfuric acid, prechilling, dry heat treatments improved to some extent these traits. The results of this study suggested using cream seeds treated with manual scarification or GA_3 to get high yield of teosinte. Moreover, white seeds can be stored under open-air conditions and be used as carryover seeds or sowing white seed early in the growing season to its break dormancy.

INTRODUCTION

Seed dormancy is caused by some blocks to germination within the imbibed seed and its germination depends on the relief of this dormancy. Dormancy may simply occur because the embryo is immature, but seed may be dormant in the sense that germination is blocked physiologically rather than by embryo immaturity (Baskin and Baskin, 1989). Seed dormancy is determined by both genetics and environment and is conferred by morphological and physiological factors including seed coat, substances in seed that protect and covering (flavenoids and lipid) and plant hormone balance (North *et al.,* 2010). Teosinte has survived as wild plant because the pistillate spike breaks up at maturity to disperse the kernels, which unlike maize, kernels are protected in heavy cellulose-lignin structure called fruit cases. Fruit cases are composed of hard segments of the rachis of the spike, and lignified outer glumes and may be black, gray with black or ivory white (Beadle, 1971). Teosinte seed germination and subsequent field emergence didn't reach the optimum levels that are lead to decreasing plant density and the total yield. Some researchers stated that annual teosinte seed is probably dormant when harvested and require after ripening a period for breaking dormancy.

To accelerate breaking seed dormancy, hormones have been applied in several studies (Chang and Sung, 2000 and Keshtkar *et al*., 2008a). Gibberellic acid (GA_3) is one of the hormones proposed to control primary dormancy by inducing germination (Iglesias and Babiano, 1997). Plant growth regulators such as GA_3, chemicals such as sulfuric acid (Nadjafi *et al*., 2006 and Rahnama-Gahfarokhi and Tavakol-Afshari, 2007) and mechanical scarification, hot water (Hermansen *et al*., 2000) have been recommended to break dormancy and enhance germination. Moreover, chemicals may also be used to cause degradation of the seed coat. Soaking hard-coated seed in concentration or diluted sulfuric acid removes seed impermeability (Copeland and McDonald, 1985).Scarification aims to abrade the seed coat so as to permit water absorption. Physical scarification may be performed by hand, especially for laboratory purposes, or by the use of specially designed machines. Piercing, chipping, nicking or filing the testa of individual seeds with a mounted needle, knife, hand file or abrasive paper is a technique especially suitable for small quantities of seeds (ISTA, 1981). The best method of breaking teosinte seed dormancy is by mechanical scarification, suggesting that germination inhibitors are contained in the seed-covering tissues López *et al.* (2011). Prechilling method was used according to ISTA (2011).

The aim present investigation was to determine the effect of some seed treatments i.e. sulfuric acid scarification, prechilling, dry heat, mechanical scarification, soaking in GA_3 treatments on seed germination, seedling vigor, field emergence, fresh and dry yields of the three seed colour degree i.e. (white, cream and mixed between white and cream) of local population Damietta teosinte.

MATERIALS AND METHODS

Laboratory and field experiments were conducted at the Laboratory of Seed Technology Research Unit, Mansoura and Experimental Farm Station of El-Serw, Damietta Governorate, during the seasons of 2014 and 2015. Seed samples were supplied by Forage Crops Research Dept. at Field Crops Research Institute .The Seeds were cleaned from dust husk and any inert materials then separated to the three colour degrees (Table, 1), then the seeds were subjected to laboratory tests to determine the weight of 100 seeds germination percentage and tetrazolium (TZ) staining value in accordance with the procedures out lined by the International Seed Testing Association (ISTA, 2011).

Pre-sowing seed treatments were included acid scarification where Seed samples were soaked in sulfuric acid 10%, 20% and 30% concentrates for one and two hours. Thereafter, the seeds were rinsed several time in clean distilled water and tested for germination. Moreover, Gibberellins treatment was mixed with distilled water and made different concentrations. The seeds of different colours were soaked in three GA_3 concentrations (500, 1000 and 1500 ppm) for 24 hours. Prechilling treatment was done by placing the seeds in contact with the moisture substrate and kept at low temperature

(3^{o}C±2) for 7 days before they removed to normal temperature gradually and tested for germination. For preheating treatment, seed samples were heated at temperature 50^{o}C with free air circulation for a period of 24 hours before they subjected to germination test (ISTA 2011). Finally, manual scarification was used by removal of seed covering tissue using cutter pliers to expose the main structure of the embryo. Precaution was taken to scarify the seed coat at the suitable place in order to avoid damaging the embryo.

Germination rate was defined according to Bartlett (1937) as follows:

$$\text{Germination rate} = \frac{a + (a + b) + (a + b + c) + \ldots\ldots + (a + b + c + m)}{n\,(a + b + c + \ldots\ldots\ldots\ldots + m)}$$

Where (a, b and m) number of seedlings emerged at the first count of germination test, second count and final count and (n) is the number of counts.

Mean germination time was calculated by the following equation:

$$\text{Mean germination time} = \frac{(N_1 \times T_1) + (N_2 \times T_2) + (N_3 \times T_3) + (N_1 \times T_4)}{N_1 + N_2 + N_3 + N_4}$$

N_1, N2, N3 and N_4 = First, second, third and four counts, respectively. T_1, T_2, T_3 and T_4 = Time of first, second, third and four counts, respectively)

Shoot and root lengths (cm) of seedling were determined at the final count of germination test where 10 normal seedlings from each replicate were taken randomly to measure the shoot and root lengths

Seedling dry weight was taken as an average of ten normal seedlings from each replication according to Kirshnasamy and Seshu (1990).

The best result of each treatment was selected and submitted to statistical analysis.

Field characters: The experimental was laid out a randomized complete block design (RCBD). The plot area was 6 m^2 (2X3m). The preceding winter crop was berseem in both seasons. Field emergence was estimated by using germinated seed/total planted seed**.** Forage fresh weight was estimated (kg/plot). Dry matter was also determined using the random samples of known weight (250 g) taken from each plot, These samples were dried at 70^{o}C for 15 hours and then at 105^{o}C till a constant weight, Then dry forage yield (kg/plot) was calculated.

Collected data for each season were statistically analyzed by the technique of analysis of variance and the least significant differences (LSD) of treatments (Gomez and Gomez, 1984). Bartlett test was done to the homogeneity of error of variance. The test significant for all laboratory experiment traits, thus the data were combined for these traits only.

RESULTS AND DISCUSSION

Laboratory characters:

The results in Table 1 showed that germination percentage, germination rate (GR) and mean germination time (MGT) as affected by the three colour degrees. The highest germination capacity, germination rate and mean germination time values were recorded from seed have cream colour (64%, 0.682 and 2.6 days), whereas, the lowest values resulted from white seed (49%, 0.452 and 4.1 days). The mixed (between cream and white) was ranked secondly. The decrease in germination percentage, germination rate and germination time may be due to teosinte fruit cases being closely associated with absence or poor development of embryos. These findings were associated with Margarita (1981) and Amany, Sallam and Hoda Emama (2014). Also, GA_3 affects physiological and metabolic activities of seeds, results in early germination (Chuanren *et al.,* 2004).

Table 1: Seed weight of the three colour degrees on germination%, germination rate and mean germination time of teosinte seed.

Weight (g)			Germination %			Germination Rate			mean germination time (day)		
White	Cream	mixed	White	Cream	Mixed	White	Cream	Mixed	White	Cream	Mixed
3.09	4.23	3.75	49	64	55	0.452	0.682	0.581	4.1	2.6	3.5
0.31			5.0			0.017			0.3		

Data in Table 2 showed the effect of pre-sowing treatments e.g. Sulfuric acid (10% for one hour); gibberellic acid (1500 ppm) for 24 hours; prechilling ($3^oC\pm2$) for 7 days before it removed to normal temperature gradually; dry heat method 50^oC with free air circulation for a period of 24 hours; manual scarification on germination, germination rate and mean germination time of teosinte seeds. The highest germination % was resulted from the seed treated with manual scarification (79%) and GA_3 (69%). Moreover, the highest germination rate was observed for the same treatments (0.835). The untreated seeds recorded the lowest mean germination time (0.426) which might be due to the strong inhibitory effect of seed coat caused by several possible mechanisms, including mechanical constraint, preventing of water and oxygen uptake and production of chemical inhibitors. The results are in agreement with those reported by Taiz and Zeiger (2002). Moreover, GA_3 has been exogenously applied as a substitute for stratification and increased germination in many plant species (Keshtkar *et al*., 2008b). López *et al.* (2011) reported that the best method of breaking teosinte seed dormancy is by mechanical scarification, suggesting that germination inhibitors are contained the seed covering tissue.

Table 2: Effect of pre-sowing seed treatments on germination%, germination rate and mean germination time of teosinte.

Treatments	Germination %	Germination rate	mean germination time (day)
H_2SO_4 10% 1hour	57	0.575	2.9
GA_3	69	0.648	2.1
Prechilling	48	0.646	3.8
Dry heat	34	0.303	4.3
Manual Scarification	79	0.835	2.1
T.Z.	82	---------	---------
Control	27	0.426	5.3
LSD at 0.05	4	0.0433	0.2

TZ detected only with germination percentage

Data presented in Table 3 illustrated significant effect of seed colour degrees on shoot, root lengths and seedling dry weight. The highest shoot and radical lengths was recorded from cream seeds (14.07, 6.51 cm and 1.436 g). Meanwhile, the lowest values were observed in white seeds. Some knowledge about seed development and physiological maturity helps to understand seed quality in term of germination and vigor. Seed maturity will be under developed and store less food reserve as compared to those at physiological maturity (Deshapande *et al.,* 1991). These findings are in harmony with Fridborg *et al.* (2001) who reported that exogenous application of GA_3 can suppress the activity of shoot internode and GA_3 intensive genes and hence leads to elongation growth of shoots.

Table 3: Effect of the three colour degrees of teosinte on shoot length (SL cm), root length (RL cm) and shoot dry weight (SDW g) of teosinte.

Shoot length (cm)			Root length (cm)			Seedling dry weight (g)		
White	Cream	Mixed	White	Cream	Mixed	White	Cream	Mixed
8.56	14.07	12.32	4.15	6.51	5.73	0.831	1.436	1.268
0.74			0.63			0.106		

Table 4 showed the effect of the interaction between presoaking treatments and seed colour degrees, on germination (%), germination rate and mean germination time. The highest germination percentage was achieved when cream seeds treated with manual scarification (85%) followed by cream seeds treated with gibberellins (76%). While, the lowest germination value was achieved from untreated white seeds (17%) and treated white seeds with dry heat (18%). Moreover, germination rate recorded the highest with cream seeds when using manual scarification method and the lowest one was recorded from control treatment with white seeds. On the other hand, mean germination time was the highest with cream seeds under the manual scarification (1.00 day), whilst the control treatment with white seeds achieved the lowest value (5.7 days).

Table 4: Effect of pre-sowing treatments and seed colour degrees on germination (%), germination rate and mean germination time of teosinte seeds.

Treatments	Germination %			Germination Rate			mean germination time (day)		
	White seeds	Cream seeds	Mixed seeds	White seeds	Cream seeds	Mixed seeds	White seeds	Cream seeds	Mixed seeds
$H_2SO_4$10%one hour	43.00	70.00	58.00	0.425	0.728	0.571	3.53	2.33	2.700
GA_3	63.00	76.00	68.00	0.470	0.769	0.698	2.367	1.867	2.133
Prechilling	45.00	52.00	45.00	0.608	0.687	0.650	4.500	2.767	4.067
Dry heat	18.00	46.00	36.00	0.176	0.434	0.297	5.400	3.033	4.467
Manual Scarification	73.00	85.00	79.00	0.737	0.922	0.845	3.300	1.00	1.967
T.Z.	76.00	87.00	82.00	-----	-----	-----	-----	------	-----
Control	17.00	38.00	25.00	0.297	0.555	0.426	5.700	4.767	5.367
LSD at 0.05	7.00			0.106			0.351		

TZ was done with germination percentage only

Table 5 showed that GA_3 and Manual scarification treatments of cream seeds recorded the highest shoot length (20.0 cm and 18.9 cm); mixed seed recorded (16.9 and 18 cm) whereas untreated white seed produced shorter shoot length (3.3 cm). Other treatments (prechilling and drying) improved slightly shoot length and the better improvement achieved from cream seed. The results of root length and shoot dry weight showed the same trend as those of shoot length, where the lowest values resulted from untreated seeds particularly, those of white colour seeds. The coating tissues have been hypothesized to severe as a physical barrier to germination of dormant seeds of teosinte as they may contain germination inhibitors. Exposure seed to scarification or gibberelic acid have been suggested as treatments to break dormancy in teosinte (Mondrus, 1981 and Taba *et al.* 2004). Also, scarification plays a role in permeability to water and induce the oxygen availability. (Copeland and McDonald, 1985)

Table 5: Effect of the pre-sowing seed treatments and seed colour degrees on shoot length "cm", root length "cm" and seedling dry weight "g" of teosinte.

Treatments	Shoot length (cm)			Root length (cm)			Seedling dry weight (g)		
	White seeds	Cream seeds	Mixed seeds	White seeds	Cream seeds	Mixed seeds	White seeds	Cream seeds	Mixed seeds
H_2SO_4 10% one hour	10.1	14.4	13.1	4.3	6.1	5.5	1.000	1.540	1.460
GA_3	12.4	20.0	16.9	7.1	9.7	8.8	1.116	2.081	1.810
Prechilling	6.3	13.3	11.2	3.4	5.8	6.8	0.660	1.365	1.05
Dry heat	4.0	11.8	9.5	1.6	5.1	3.9	0.194	1.174	0.920
Manual Scarification	15.36	18.9	18.0	6.3	9.1	8.6	1.630	1.870	1.810
Control	3.3	6.1	5.2	2.3	3.3	3.0	0.382	0.581	0.483
LSD at 0.05	1.1			0.9			0.149		

Field characters:

Field emergence

Data in Table 6 show the effect of pre-sowing treatments and seed colour on field germination. High field emergence was recorded from manual scarification method and cream seed (74%) followed by GA_3 treatment with the same colour (68%), whilst the lowest value was observed from control treatment of white seed. These findings agreed with those obtained by Mondrus (1981) and Taba *et al.* (2004). They reported that exposure seed to scarification or gibberellic acid have been suggested as valuable treatment to break dormancy in Teosinte seed.

Table 6: Effect of pre-sowing seed treatments and seed colour on field emergence (%) of teosinte.

Treatments	Field emergence %		
	Cream seeds	White seeds	Mixed seeds
H_2SO_4 10% one hour	40	65	55
GA_3	57	68	63
Prechilling	47	41	42
Dry heat	16	42	32
Manual Scarification	60	74	71
Control	14	34	15
LSD at 0.05	4.0		

2: Fresh and dry weights:

Data in Table (7) shows the effect of pre-sowing treatments of colour of seed teosinte on the three individual and total fresh cuts. The highest fresh weight cuts of teosinte were recorded from cream seeds treated with manual scarification followed by GA_3 (4.9 and 3.2; 13.2 and 12.0; 8.2 and 13.0 and 26.2 and 22.8 and 22.8 kg/plot) for cut1, cut2, cut3 and total cuts, respectively. While, the lowest values of fresh weight resulted from untreated white seeds (0.8, 3.6, 3.9 and 8.3 kg/plot) for cut1, cut2, cut3 and total cuts, respectively.

Table 7: Effect of pre-sowing seed treatments and seed colour on fresh weight (kg/plot) at different cuts and total fresh weight (kg/plot) of teosinte (combined analysis).

Treatments	Fresh weight cut1			Fresh weight cut2			Fresh weight cut3			Total Fresh weight		
	White	Cream	Mixed	White	Cream	Mixed	White	Cream	mixed	White	Cream	mixed
H_2SO_4 10%one hour	1.5	1.9	1.7	7.2	11.4	9.7	3.0	5.3	4.2	11.7	18.7	15.6
GA_3	2.5	3.2	2.8	9.3	12.0	10.5	5.3	7.7	6.2	17.1	22.8	19.5
Prechilling	2.0	3.1	2.4	8.1	11.6	9.2	3.6	6.2	4.6	13.6	20.9	16.2
Dry heat	1.3	2.7	2.3	7.3	10.6	8.6	4.5	7.3	5.3	13.1	20.7	16.2
Manual Scarification	3.6	4.9	3.2	9.2	13.2	10.2	4.1	8.2	5.2	16.9	26.2	18.6
Control	0.8	1.3	1.1	3.6	8.6	4.0	3.9	5.0	4.7	8.3	14.9	9.8
LSD at 0.05	0.9			1.7			1.5			2.4		

Data in Table (8) shows the effect of pre-sowing treatments of seed colour on dry matter in various and total cuts. Manual scarification as pre-sowing treatment for cream colour achieved the highest dry water (0.733 kg/plot). White seed subjected to the same treatment recorded (0.647 kg/plot) which over passed the dry weight produced from other treatments from the first cut. These results that the effect of seed treatment is considerably high as compared to that of seed colour. However, the dry weighs from letter cuts were higher from the same colour seed (white) treated with GA_3 (1.687,1.820 and 3.960 kg/plot, at the second, third and total cuts, respectively). The lowest dry matter in the first cut was resulted from white untreated seeds. The results of total dry matter (kg/plant) as affected by pre-sowing treatments and colour degrees are outlined by López *et al.* (2001) they reported that seed covering tissue are the most important barriers to germination. Without removal of the rachis tissues and the lemma and palea chaff for several populations the seed will germinate after 8-20 months. Regardless the seed colour, GA_3 application to the seed increasing cell wall extensibility lading to elongation of internodes (Rahman *et al.*, 2004).

Table 8: Effect of pre-sowing seed treatments and seed colour on dry weight (kg/plot) at different cuts and total dry weight of teosinte (combined analysis).

Treatments	Dry weight cut1			Dry weight cut2			Dry weight cut3			Total Dry weight		
	White	Cream	Mixed	White	Cream	mixed	White	Cream	mixed	White	Cream	mixed
H_2SO_4 10% one hour	0.243	0.363	0.300	1.417	2.010	1.693	1.303	1.650	1.217	2.960	4.020	3.210
GA_3	0.457	0.597	0.637	1.687	2.073	1.803	1.820	2.357	1.993	3.960	5.030	4.430
Prechilling	0.383	0.583	0.550	1.240	1.957	1.240	1.413	2.130	1.420	3.040	4.670	3.840
Dry heat	0.250	0.543	0.467	1.450	1.227	1.770	1.430	2.110	1.313	3.130	3.880	4.180
Manual Scarification	0.647	0.733	0.607	1.480	2.183	1.873	1.767	2.230	1.940	3.890	5.150	3.720
Control	0.197	0.247	0.240	1.070	1.803	1.197	1.003	1.350	0.910	2.270	3.400	2.350
LSD at 0.05	0.138			0.257			0.457			0.458		

The previous results indicated the importance of subjecting teosinte seed to special treatment before sowing in the field, simply because the seed may be dormant. Such dormancy forbids the seed to germinate, however in the seed lots used in the present research, some seeds were capable of germinating and other seed of the same lot didn't .The reason might be due to seed covering tissue which related to seed maturity at harvesting the crop (i.e. embryo is immature), but germination of teosinte seed is blocked because of physiological reason rather than by embryo immaturity and require after ripening a period for breaking dormancy. (Baskin and Baskin,1989). The variation in colour of seed is mainly genetically controlled and the plant breeder should take into consideration this fact when selection the breeding material. The results of this study suggested using cream seeds treated with manual scarification or GA_3 to get high yield of teosinte .moreover, further research is need to answer the question" if white seed is stored under open-air conditions and be used as carry over seed will break its dormancy". or " if sowing white seed early in the growing season will improve its germination under field conditions.

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معاملات كسر السكون فى البذور وعلاقتها بالإنبات وقوة البادرة والمحصول فى الذرة الريانة

شريف عبد الغنى أبوالجود

قسم بحوث محاصيل العلف، معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية

أقيمت تجربتان معملية وآخرى حقلية بوحدة تكنولوجيا البذور بالمنصورة ومحطة البحوث الزراعية بالسرو خلال عامى ٢٠١٤ و ٢٠١٥.

تهدف هذه الدراسة إلى تحديد تأثير الإختلاف فى لون البذور ذات التركيب الوراثى المتماثل وكذا نوع معاملات كسر السكون ما قبل الزراعة على انباتها تحت ظروف المعمل ونمو النباتات فى الحقل والمحصول الاخضر والجاف فى الذرة الريانة . اشتملت الدراسة على ثلاث درجات من اللون وهى الأبيض والكريمى والخليط من الأبيض والكريمى فى عشيرة دمياط. أشارت النتائج ان البذور ذات اللون الكريمى أعطت أعلى نسبه إنبات فى المعمل وقوه بادرات مقارنة بالبذور ذات اللون الأبيض بينما أعطت البذور الخليط من اللونين (الابيض والكريمى) قيم وسطية بين النوعين. وأشارت النتائج إلى أن البذور التي أجرى لها خدش ميكانيكي حققت أعلى نسبه إنبات ومعدل إنبات مقارنة بالبذور التى أجريت لها معاملة بالجبرلين وحامض الكبريتيك والبرودة والتجفيف مع الهواء الساخن. كما أوضحت النتائج أن البذور ذات اللون الكريمى التي أجرى لها خدش ميكانيكي أعلى نسبه إنبات (تكشف حقلى) وقوة بادرة ونمو خضرى فى الحقل ووزن طازج ووزن جاف تحت ظروف الحقل تلاها البذور المعاملة بالجبرلين وذلك خلال الحشات الاولى والثانية والثالثة والمحصول الكلى. بينما كانت اقل القيم قد نتجت من البذور البيضاء غير المعاملة . وتقترح الدراسة استخدام بذور الذرة الريانه ذات اللون الكريمى مع الخدش الميكانيكي أو النقع في الجبرلين حتى يمكن الحصول على أعلى محصول من الذرة الريانه.