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in

Delgado I. (ed.), Lloveras J. (ed.).
Quality in lucerne and medics for animal production

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 45

2001
pages 137-139

Effect of temperature on germination and hard alfalfa seed

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SUMMARY – The purpose of the thesis was to find out the influence of different temperature treatments on the reduction of percentage of hard seeds in three alfalfa varieties (Osjecka 10, Osjecka 88 and Slavonka). The trial comprised five different treatments: control treatment; 5 days +7ºC; 5 hours +40ºC; 1 hour +80ºC; 2 times 2 hours -80ºC and two hours at +22-23ºC in between. The two treatments, +80ºC and -80ºC were very uniform and efficient since they reduced the percentage of hard seeds in comparison with the control by 71-91% while their influence on other germination parameters was completely different. In the +80ºC treatment, up to 25 times more dead seeds and up to 5 times more abnormal seedlings, if compared to -80ºC, were found and, on the other hand, very low values for germination energy. The treatment with -80ºC had only positive effects that were reflected in the highest values for germinative energy and germination. The temperature +40ºC did not have an important influence on the percentage of hard seeds while the treatment with prechilling was found ineffective.

Key words: Alfalfa (*Medicago sativa* L.), varieties, temperature, hard seed.

RESUME – *"Effet de la température sur la germination de la luzerne et le taux de semences dures". L'objectif de cette thèse était de découvrir l'influence de différents traitements thermiques sur la réduction du pourcentage de graines dures des trois sortes de luzerne (Osjecka 10, Osjecka 88 et Slavonka). L'épreuve consistait en 5 différents traitements : contrôle ; 5 jours +7ºC ; 5 heures +40ºC ; 1 heure +80ºC ; 2 fois 2 heures -80ºC et entretemps deux heures à +22-23ºC. Les traitements à -80ºC et +80ºC étaient très égalés et efficaces, étant donné que le pourcentage de graines dures en comparaison avec le contrôle a été diminué de 82-84%, alors que leur influence sur les autres paramètres de la germination a été complètement différente. Chez le traitement avec +80ºC en comparaison avec -80ºC, il y avait jusqu'à 25 fois plus de graines mortes et jusqu'à 5 fois plus de germes anormaux, et, d'autre part, de très basses valeurs pour la germination et pour l'énergie germinative. Le traitement à -80ºC n'avait aucune influence négative, mais seulement positive, ce qui se reflète dans les plus grandes valeurs pour l'énergie de la germination et la faculté germinative. La température de +40ºC n'avait aucune influence importante en ce qui concerne le pourcentage de graines dures, tandis que le traitement avec pré-refroidissement était tout à fait inefficace.*

Mots-cles : *Luzerne, dormance, graines dures, germination, viabilité des graines.*

Introduction

Hard seed share in perennial leguminous plants may range up to 80% and more (Tkacenko *et al.,* 1978). There are different reasons for hard seed occurrence. Temperature and precipitations in alfalfa seed maturation period followed by genetic variety properties are mostly mentioned as agents of hard seed occurrence. Hard alfalfa seed does not germinate due to seed plant impermeability having compacted cells with thickened outside cell walls usually coated by a wax layer and well developed cuticule (Kastori, 1984). Hard seed occurrence in commercial seed can be tolerated to a certain amount depending on seed purpose. Thus, Bass *et al.* (1988) claimed that market seed should not contain more than 10% of hard seed.

Hard seed reduction is possible by seed thining performed either mechanically, by temperature, by chemical agents or by other ways. Hard seed reduction in total seed by temperature was obtained by Hall *et al.* (1998), Wiesner *et al.* (1994) and Stout (1990). Seed treatment aims at achieving hard seed reduction in a total seed whereby seed quality is not lower but higher or the same compared to untreated seed.

Materials and methods

Laboratory investigations comprised three alfalfa varieties: Osjecka 10 (OS-10), Osjecka 88 (OS-88) and Slavonka. The investigation variants were as follows: (i) control seed was not subjected to the treatment (K); (ii) seed was exposed to cooling treatment for 5 days at 7ºC (PH); (iii) seed was exposed to a temperature of 40ºC for 5 hours (40); (iv) seed was first exposed to a temperature of -80ºC for 2 hours then to a room temperature for 2 hours (-80) (temperature of -80ºC was obtained by dry CO_2 and acetone); and (v) seed was exposed to a temperature of 80ºC for 1 hour (80). Each procedure was followed by determination of germination free of hard seed, germinative energy, abnormally developed germs, dead seed as well as hard seed and germination with hard seed.

Results and discussion

The highest germinative energy was achieved with all three varieties by seed freezing procedure at -80ºC whereas the lowest one by the procedure at 80ºC (Table 1), especially in variety Slavonka (1.75%). Differences were statistically justifiable.

The temperature of -80ºC obtained the highest seed germination free of hard seed and the lowest one was achieved by the temperature of 80ºC, especially in variety Slavonka (2%). Differences were statistically justifiable (Table 1).

Dead seed share was the lowest in untreated variant ranging from 0.50 to 3.50% whereas the highest dead seed share was in the variant treated by the temperature of 80ºC ranging from 30.75 to 71.50% (Table 2).

Table 1. Germinative energy and seed germination

Procedure	Germinative energy (%)			Germination free of hard seed (%)		
	OS-10	OS-88	Slavonka	OS-10	OS-88	Slavonka
1. K	55.50a	69.25a	54.00ab	61.50a	76.50a	60.25ab
2. PH	54.50a	67.75a	48.00a	59.00a	73.75a	52.75a
3. 40	58.50a	69.75a	59.25b	69.75b	79.00a	69.50b
4. -80	74.75b	80.25b	73.25c	86.25c	89.00b	82.00c
5. 80	15.25c	30.75c	1.75d	17.25d	33.25c	2.00d
LSD (0.05)	7.71	8.36	7.24	7.04	7.81	9.82

Table 2. Dead seed and abnormally developed germs

Procedure	Dead seed (%)			Abnormal germs (%)		
	OS-10	OS-88	Slavonka	OS-10	OS-88	Slavonka
1. K	0.75a	0.50a	3.50a	29.25a	19.00a	26.75a
2. PH	1.25a	1.75a	5.00a	29.75a	17.00ab	28.00a
3. 40	1.00a	1.25a	2.50a	16.75b	13.75b	18.00b
4. -80	2.50a	1.25a	3.50a	4.00c	1.75c	5.25c
5. 80	37.75b	30.75b	71.50b	7.25c	5.50c	4.50c
LSD (0.05)	10.19	5.72	8.42	4.45	4.13	5.23

Hard seed share prior to treatment, depending on a variety, ranged from 19.00 to 29.25% being confirmed by Stout (1990) that genetic variety properties affect hard seed share. Due to temperature exposure of -80ºC, hard seed share was reduced in all three varieties from 1.75 to 5.25% (Table 3). Hard seed reduction due to low temperature exposure was achieved by Stout and Langton (1991) and Wiesner *et al.* (1994).

Table 3. Hard seed and total germination

Procedure	Hard seed (%)			Total germination (%)		
	OS-10	OS-88	Slavonka	OS-10	OS-88	Slavonka
1. K	29.25a	19.00a	26.75a	90.75a	95.50a	87.00a
2. PH	29.75a	17.00ab	28.00a	88.75a	90.75a	80.75a
3. 40	16.75b	13.75b	18.00b	86.50a	92.75a	87.50a
4. -80	4.00	1.75c	5.25c	89.75a	90.75a	87.00a
5. 80	7.25c	5.50c	4.50c	24.50b	38.75b	6.50b
LSD (0.05)	4.45	4.13	5.23	6.96	6.96	7.41

Considerable hard seed reduction was attained by 80ºC temperature treatment (Table 3). Obtained results are in accordance with the statements by Tkacenko *et al*. (1978) and Bass *et al*. (1988).

Differences in total germination, with all three varieties, attained by the investigated procedures were not statistically justifiable, except in treatment at 80ºC. The highest germination was achieved on untreated variant, ranging, depending on variety, from 87.00 to 95.50% (Table 3).

Conclusions

(i) Hard seed reduction due to seed treatment at -80ºC was 91.9% in variety Osjecka 88, 86.3% in Osjecka 10 and 80.4% in Slavonka. Germinative energy and germination itself free of hard seed were increased by this procedure. Germinative energy, depending on a variety, ranged from 73.25% (Slavonka) to 80.25% (Osjecka 88) whereas germination free of hard seed amounted to 82.0% (Slavonka) and 89.0% (Osjecka 88).

(ii) Procedure with a temperature of 80ºC reduced hard seed percentage but the seed was not market valuable due to low germinative energy and germination. There were 5 times more abnormal germs and 25 times more dead seeds compared to the procedure with -80ºC temperature.

(iii) Hard seed share was reduced by 40ºC temperature, depending on the variety, from 57.26% (Osjecka 10) to 72.4% (Osjecka 88), which affected germinative energy increase as well as germination free of hard seeds in the three varieties.

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