The Effect of Pod and Priming on Germination of Sainfoin Seed

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Abstract: Sainfoin seeds are born in single-seed pods. Pods use instead of seed in agronomic condition. The objective of this study was to evaluate influence of pod color on seed germination of sainfoin in conjunction with the effect of priming on it. Factors were including of: pods color (brown and green), priming (hydro-priming and without priming) seed pod persistency (pod shelled and unshelled). The results showed that the presence of pods at all treatments reduced germination and vigor parameters. The brown seed pods showed higher germination percentage than the greenish seed pods. The non-primed and greenish seed lot of sainfoin recorded the lowest germination percentage and germination rate. Hydro-priming significantly increased the germination rate and reduced germination time mean, T50 in both green and brown sainfoin seed. Based on the result of germination test it can be suggested to use as a carry-over seed it would be suitable to use the brown seed pods. Also results of the present study suggest that hydro-priming is very effective tool for seed invigoration enhancement unshelled seeds (pods) of sainfoin.

Key words: Sainfoin, Pod color, Priming, Seed germination

INTRODUCTION

Sainfoin (Onobrychis viciifolia Scop.) is important forage legumes in the Mediterranean, western Asia, Europe, western USA and Canada (Miller and Hoveland, 1995; Frame et al., 1998). This species is a short-lived perennial with important traits due to its wide ability to adapt to various conditions. Sainfoin as forage crop has high nutritional value and its forage has non-bloating properties (Waghorn et al., 1990. McMahon et al., 1999). Feeding sainfoin to lambs has also been shown to provide equivalent levels of performance to feeding Lucerne (Medicago sativa L) (Karnezos et al., 1994).

Sainfoin is a perennial forage legume adapted to many areas of Iran such as Feridan and Shahrekord regions. It can be used as an irrigated or dryland pasture species or for harvested forage (Peel et al., 2004). In the context of Iran agriculture, sainfoin have capability for increasing use in the future based on its attributes of faster growth in early spring, cold and drought tolerance, nitrogen (N) fixation, and it has several nutritional advantages, including preventing bloat in ruminants (Peel et al., 2004. Sheldrick et al., 1995. Frame et al., 1998). However, future use of sainfoin is limited by the lack of some agronomic information.

Pod shattering has been a major constraint to seed production of sainfoin. Delayed seed harvest may increase seed losses due to pod shattering; however, early harvest may influence seed germination due to maturity of fruit or seeds. Commonly seed lots of sainfoin are combination of greenish to brown pod colors (Thomson, 1951). Little research attention has been give to the effect of maturity stage in sainfoin on the ability of seed germination.

Unlike alfalfa, sainfoin seeds are born in single-seed pods (Peel et al., 2004). The pod (indehiscent fruit) is bean-shaped and bilaterally compressed, and it has a rough net-veined appearance. Pods use instead of seed at sowing time. The seed itself is kidney-shaped, varying from yellowish green to dark brown or olive in color.
The seed is much larger than seed of other common forage legumes (Thomson, 1951).

Seed can be planted with pods removed (hulled) or intact (unhulled); however, pod removal is generally advantageous but injury to seed is available irrespective of cost. As the germinating seedling emerges from the pod, the radicle can be injured and infected by *Alternaria* and *Fusarium* spp. pathogens (Ditterline and Cooper, 1975). Additionally, the pods slow water imbibition and contain water-soluble inhibitors that slow germination (Carleton et al., 1968). In Iran sainfoin is planted only with pods intact. However its presence limited germination and the slow and non-uniform seedling growth, uneven crop stand and high weed infestation and farmers need use large amount seeds in planting time for obtaining of favorite plant density.

Seed vigour enhancement treatments have been proven to be very effective to achieve rapid and uniform seed germination of several vegetable species (Taylor et al., 1998. Ghassemi-Golezani and Esmaeilpour, 2008) and field crops (Ismail et al., 2005. Lee et al., 1998. Basra et al., 2002; 2003; 2005. Farooq et al., 2005; 2006. Moradi and Younesi, 2009) and cover crop as well as pasture grass (Snapp et al., 2008; Tavili et al., 2009). These treatments include priming, hardening, humidification, growth regulators, pre-germination and dry heat treatments.

Hydro-priming also called wetting and drying or hydration-dehydration refers to soaking in aerated water and drying at 15-25°C (Mc Donald, 2000. Artola et al. 2003). The beneficial effects of seed hydro-priming have been attributed to pre-enlargement of the embryo (Austin et al., 1969), biochemical changes like enzyme activation (Lee and Kim, 1999; 2000. Basra et al., 2005), improvement of germination rate particularly in old seeds (Lee et al., 1998) and increase of invigoration seeds and grain yield (Ghassemi-Golezani et al., 2008). Hardening (repeated hydration-dehydration) treatment of fine rice for 24 h proved to be the best for vigor enhancement (Basra et al., 2003; 2005).

However, to our knowledge no studies have been reported on the effect of priming on sainfoin due to seed pod, despite the low seed vigor prevalent in this crop therefore the objective of work reported here was to evaluate the impact of seed priming in sainfoin seeds on germination performance and seed vigor. This study was conducted to determine whether seed pod really affects seed germination and vigor parameters and whether effect of persistency and color of seed pod in sainfoin was affected by priming treatments?

**MATERIAL AND METHOD**

**Seed Preparation:**

The seeds (pods) were visually selected on the basis of pod color difference while shining white light on the pods placed on a dark background and divided into green (include: green, pal green-yellow) and brown (include: cinnamon-buff, wood brown, brown) components. Each color was divided two sub samples, one part used for hydro-priming and second part used for non-priming treatments.

The experiment was conducted on seeds collected from Boldaji ecotype in Iran. Mean 1000 unshelled and shelled seeds weight were 23.4, 17.7 g respectively.

**Hydro-priming Method:**

Sub samples of two pods color (25 g) were soaked in 500 mL of aerated distilled water for 24 h at 25±1°C (this treatment selected from preliminary tests), water replace every 3 h, followed by air-drying under shade. The seeds were dried in 3 d at ambient room conditions (25 ± 1°C) in order to bring the seeds to their approximate original moisture content.

**Germination Tests:**

Different categories pods (primed and non-primed of green and brown color) divided two parts, one part used for unshelled seed germination and second part at first was shelled by hand and used for hulled seed germination. All categories of sainfoin seeds were tested for germination potential. All the seeds were washed with distilled water and then treated with Rovral TS 1 g kg⁻¹ to retard saprophytic fungi. Four replications of 30 seeds for each pod size category were placed in covered Petri dishes containing one layer of Whatman 1 filter papers moistened with 4 ml distilled water. Petri dishes were placed in a germination chamber at 25°C with 12 h fluorescent light and 12 h dark. Seeds were identified as germinated when the radicle protruded. The Petri dishes were arranged in a completely randomized design (CRD) with four replications. Counts of germinated seeds were made daily, starting on the first day of imbibition and terminated when maximum germination was achieved. Normal seedlings were recorded for calculating germination percentage (GP) at last count.
The time to reach 50% germination ($T_{50}$) of final germination was calculated according to the following formulae (Eq. 1) of Coolbear et al. (1984), modified by Farooq et al. (2005):

$$T_{50} \text{(in days)} = t_i + \left[ \frac{\left\{ (N/2 - n_i) n_i - n_j \right\}}{(t_j - t_i)} \right]$$

$$T_{50} \text{(in hours)} = T_{50} \text{(in days)} \times 24$$  \hspace{1cm} (Eq. 1)

Where $N$ is the final number of germination and $n_i$, $n_j$ cumulative number of germinated seeds by adjacent counts at times $t_i$ and $t_j$ when $n_i < N/2 < n_j$.

Mean germination time (MGT) was calculated using the relation (Eq. 2):

$$MGT = \frac{\Sigma(TN)}{\Sigma N}$$ \hspace{1cm} (Eq. 2)

Where $N$ is the number of daily germinated seeds, and $T$ is the number of days after the trial started (Ellis and Roberts, 1980).

Germination index (GI) was calculated as described in the Association of Official Seed Analysts (1983) as the following formulae (Eq. 3):

$$\text{No. of germinated seeds at first count/days of first count +......+ No. of germinated seeds at final count/days of final count}$$ \hspace{1cm} (Eq. 3)

Statistical Analysis:

For statistical analysis, a factorial experiment ($2 \times 2 \times 2$) with completely randomized design with four replications was used. Factors were including of: seed pod color (brown and green), seed pod persistency (shelled and unshelled) and priming (hydro-priming and without priming).

Variance analysis was based on the ANOVA procedure by software MSTATC. Differences among treatments means were estimated using the Duncan's multiple range test at the 5% probability level.

RESULTS AND DISCUSSION

ANOVA analysis showed that the effect of factors pod color, priming and persistency of pod for GP, GI, MGT, $T_{50}$ were completely significance ($P<0.01$) (Table 1).

| Table 1: ANOVA analysis for pod color, seed priming and persistency of pod effects on germination parameters of sainfoin seeds |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | GP              | GI              | MGT             | $T_{50}$        |
| Pod color (P.C) | **             | **             | **             | **             |
| Prime (P)       | **             | **             | **             | **             |
| P.C.P            | ns             | *              | ns             | ns             |
| Pod Persistency (Pod) | **      | **             | **             | **             |
| P.C.Pod          | ns             | ns             | *              | ns             |
| P*Pod            | *              | **             | **             | *              |
| P.C.P.Pod        | ns             | ns             | ns             | ns             |
| *significant at the 0.05 probability level
** significant at the 0.01 probability level
ns = not significant at $P<0.05$

| Table 2: Mean comparison of pod color, seed priming, and persistency of pod effects on germination parameters of sainfoin seeds |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | GP              | GI              | MGT             | $T_{50}$        |
| Pod color       |                 |                 |                 |                 |
| Brown           | 72.4a           | 12.3a           | 2.2b            | 38.6b           |
| Green           | 41.3b           | 6.9a            | 2.7a            | 47.8a           |
| Prime           |                 |                 |                 |                 |
| Non-priming     | 50.3b           | 6.9b            | 2.8a            | 55.7a           |
| Hydro-priming   | 63.9a           | 12.3a           | 2.0b            | 30.7b           |
| Pod             |                 |                 |                 |                 |
| Unshelled pod   | 44.9b           | 7.1b            | 2.8a            | 48.6a           |
| Shelled pod     | 69.1a           | 12.1a           | 2.1b            | 37.9b           |

Means not sharing the same letters differ significantly at $P<0.05$

GP= germination percentage
GI= germination index
MGT= Mean germination time
$T_{50}$= Hours taken to get 50% germination

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Tests were carried out to determine whether there was any difference in germination capacity between the green and brown fruits (pods). In each case the brown fruits gave a higher germination, the mean difference of 31.1% being significant at P<0.05 (Table 2).

At all cases, unshelled pods germinated lesser and were slower to germinate than shelled pods (Table 2). No significant differences (P<0.05) were detected in interaction between seed pod color and priming for GP, MGT and T_{50}. In contrast, effects of pod persistency and seed priming on all trials were significance (Table 1).

Hydro-priming had significant effect on the germination in both green and brown seed pods (Tables 1 and 2). More uniform and earlier germination was noted in hydroprimed shelled and unshelled pods as is evident from MTG and T_{50} values. Germination index data support the trend of other germination parameters. Therefore priming alliviates partly negative effects of pod on seed germination in sainfoin.

Interaction between pod* priming was significant for all germination parameters (Table 1). Irrespective of seed pod color, priming increased GP and GI in unshelled pods, 17.8 % and 6.5 in days, but in shelled seeds increasing of GP and GI by hydropriming was 8.6% and 4.5 in days (Figures 1 and 2). Also decrease of MGT and T_{50} by priming treatment was larger in unshelled than shelled seeds (Figures 3 and 4).

However it is worth noting that although hydro-priming resulted in increased germination accompanied to more synchronous emergence in both shelled and un-shelled seeds, and both seed lots (green or brown), but unshelled pods from both seed lots showed greater response than shelled pods.

**Discussion**

The color of sainfoin fruits has been variously described by Thomson (1951). The color as dark grey to grey green and as papery brown. In good samples the fruits are brown and that very pale yellow or greenish fruits are defective or unripe. In good ripe samples the seed pods are fairly bright and the color is a dark straw or a light reddish brown. Very dark and dull looking seeds should be avoided and also pale yellowish-green seeds. The former being the evidence of old age, the latter of immaturity. The ripe pods are fairly bright
reddish brown or dark straw. The final color of fruits is to a large extent determined by the degree of ripeness at the time of harvest. The color of sample in bulk depends on the proportions of different colored fruits it comprises. It was found impracticable to separate the fruits of a sample into the various color classes, but they could be divided with little difficulty into two groups, fruits showing at least some green color and fruits showing no green color (Thomson, 1951).

In this research, greenish fruits were found to have a germination capacity about 31% less than non-green fruits. Thomson (1951) also reported non-green fruits significantly gave a higher germination than green fruits. The number of hard seeds tended to be greater in the green fruits, but the difference is not significance. Color selection resulted in increased seed germination in sainfoin. All the brown colored seed pods showed high germination percentage and greenish seed pods showed poor germination. The seed pods of the sainfoin cultivars turned brown after physiological maturity. There was clear relationship between seed pod color and seed germination, and therefore color selection may be an advantage in seed processing for increasing of seed quality. Owing to uneven ripening it is impossible to harvest all the fruits at the same stage of ripeness, but the ideal to aim at are to harvest when as many fruits as possible have lost their greenish color.

Seed pod color has been reported to play a role in germination, as seed pods attain their specific color at physiological maturity common vetch (Vicia sativa) (Samarah et al., 2004). Effect of maturity stage on seed germination has been studied for other legume crops (Miles et al., 1988. Hamid et al., 1995). Maximum germination in soybean (Glycine max L) was attained when seeds were harvested at maximum seed dry weight (physiological maturity) (Miles et al., 1988). In several mungbean (Vigna radiate L) genotypes, the germination increased as the seeds matured, reaching the maximum at physiological maturity (Hamid et al., 1995). The previous studies indicate that seeds acquired the ability to germinate at a specific development stage.
There are condensed tannins in sainfoin plant (Waghon et al., 1997). It is possible that contributed to the rigidity of cell structure, thereby reducing permeability. Greenish colored seed pods of sainfoin were shown to have reduced germination and as seed pod color became darker, seeds showed higher, faster and more uniform germination. Maybe color change of pod be associated with reduction of tannins that contributed to enhancement of seed permeability and for this reason brown fruits germinated better than green fruits.

The effect of the pod upon germination was previously studied by Thomson (1951). He concluded that lower germination was due to the failure of the radicle from weaker seed to penetrate the pod. He also concluded that a water-soluble inhibitor was not present in the pod. In contrast, Cavazza (1950), cited by Oppenheimer (1960), found that the sainfoin pod hindered imbibition of water and contained inhibiting substances which delayed emergence of the radicle and hampered its development. Carleton et al (1968) investigated optimum temperatures for germination of sainfoin seed and for seedling growth in conjunction with studies of the effect of the presence or absence of the seed pod. Optimum temperature range for germination of sainfoin was from 15 to 20 °C. A higher range of optimum temperature, 20 to 30 °C, was found for seedling growth. The presence of pods at all temperatures reduced speed of germination and speed of seedling elongation. Pods on the seed slowed water absorption by 4 to 5 h. A water soluble inhibitor which slowed the speed of germination of shelled seed was detected. This inhibitor was readily removed from the pod by washing. Mechanical restriction of germination by the pod appeared to be a minor factor in the total effect of the pod on speed of germination.

At all treatments, germination and vigor parameters of shelled pods were better than unshelled pods. The decrease in mechanical restriction caused by pod could be due to several factors: namely, 1) a slow rate of water absorption, 2) the presence of an inhibitor and 3) mechanical restriction (Carleton et al., 1968. Smith,1979).

Based on the results hydro-priming probably enhances water uptake and exits inhibitors from pod in soaking period and reduces mechanical restriction of pods and therefore enhances germination and vigor parameters in unshelled pods more than shelled pods.

It may be concluded from this experiment that positive relation was found between seed pod color and all the tested germination parameters. These data support the hypothesis that mature fruits have superior performance than to non-mature fruits. The results suggest to use as a carry-over seed it would be suitable to use the brown pods. Data of the present study suggest that seed hydro-priming is very effective tool for seed invigoration in brown and green sainfoin pods, also unshelled pods respond more than shelled pods to this treatment. The possible basis of seed invigoration by priming is enhancements of water uptake and exit of pod inhibitors.

REFERENCES


