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Seed Harvesting Time Affects Seedling Emergence, Vigour and Growth: Case Study of *Rumex turcomanicus* Czerep. (*Polygonaceae*)

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Abstract

Rumex turcomanicus Czerep., belonging to family *Polygonaceae,* is one of native green vegetable in Northeast Iran. Despite the high consumption, its seed germination and dormancy aspects is inconsiderable. In order to investigate the effects of seed harvesting date on seedling emergence, vigour and growth traits of *R. turcomanicus* Czerep., the seeds were harvested at five different times, i.e., two weeks after fruiting (WAF), 6WAF, 8WAF (mature seeds), 2 month after seed ripening (MASR) and 4MASR, and were sowed immediately, at agricultural college of Ferdowsi university of Mashhad, Iran in 2012-2013. The results was showed that the highest and lowest of seedling emergence percentage, seedling emergence rate, seedling vigour index, seedling fresh and dry weight, seedling root and shoot length, total seedling length and %final normal seedling (%FNS) was obtained in the seeds which were sowed 4MASR and 2WAF, respectively. Maximum and minimum of mean emergence time (MET) was observed in the seeds which were sowed 2WAF and 4MASR, respectively. Relationship between %FNS and MET and between %FNS and emergence percentage was highly significantly negative (-0.961) and positive (+0.962), respectively. Based on the results of this experiment, it seems that the problem of germination in most of the *Rumex turcomanicus* Czerep. seeds, is probably due to a kind of morphological dormancy, which is remained in most of the fresh seeds (collected 2WAF), and eliminated in the mature seeds (collected 2MASR). Also dry seed storage of the mature seeds for two months was improved seedling emergence and vigour, significantly.

Keywords: dry storage, morphological dormancy, medicinal plant, seedling emergence, vegetable

Introduction

The family *Polygonaceae* contains about 800 species which are grouped into 30 genera and distributed widely all over the world (Stastn *et al.,* 2010). The *Rumex* genus of *Polygonaceae* has 200 species all over the word, of which 23 species are found in Iran and 10 species in Northeast of Iran (Gholami and Joharchi, 2008). Different species of *Rumex* genus were utilized due to their biological activities, such as antiviral (Cos *et al.,* 2002), antimicrobial, anti-inflammatory (Getie *et al.,* 2002) and antidiarrheal attributes (Rouf *et al.,* 2002).

Proper establishment of plant species is often dependent on the timing of germination resulted from seed responses to environmental cues (Vazquez-Yanes and Orozco-Segovia, 1996), such as light, temperature and soil moisture that are most favorable to their establishment (Baskin and Baskin, 1998). The seeds may display the dormancy cycle which is the period for maturation to germination (Baskin and Baskin, 1998). Seed germination in different species of *Rumex* genus had been studied by many researchers. Assche *et al.* (2002) were studied comparative germination ecology of nine *Rumex* species. They were reported that the seeds of *R. acetosa* and *R. scutatus* are non-dormant and can germinate immediately after dispersal. The seeds of *R. acetosella* do not germinate in the first season after dispersal, but buried seeds do not show a dormancy cycle; they might germinate in different seasons after exposure to light. Seeds of *R. conglomerates*, *R. maritimus*, *R. sanguineus* and *R. crispus* are undergo a seasonal dormancy cycle, with a low level of dormancy in winter and early spring and a deep dormancy in summer. *R. maritimus* also germinates in summer and autumn on drying muddy soils. The seeds of R. *hydrolapathum* only germinate on waterlogged soils (Assche *et al.,* 2002).

Breaking of seed dormancy in different *Rumex* species had been studied by researchers. Seed stratification of *R. acetosella* at 1 to 7°C for 20 weeks did not improve germination levels. Scarification increased the germination of dry-stored but not fresh seed. The extent of the scarification needed varies with the thickness of the seed coat. Alternating temperatures between 20 and 30°C increased the germination of *R. acetosella* seed compared with a constant 20°C (Deschênes and Moineau, 1972). Germination was greater under light conditions than in the dark in *R. acetosella*. In alternating temperatures, seed scarification reduced the light requirement (Grime and Jarvis, 1976). Also, germination was promoted by nitrate in *R. acetosella* (Grime *et al*., 1988). The effect of light quality on seed germination of two Rumex species was studied by Roberts and Totterdell (1981). R. crispus and R. obtusifolius respond positively to red or white light. Far-red

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light applied for short periods may promote or inhibit germination depending on the timing of the irradiation in relation to temperature change; but long periods of far-red inhibit germination (Roberts and Totterdell, 1981). Demirezen Yilmaz and Aksoy (2007), were reported a negative relationship between germination rate and seed age in *Rumex scutatus*. Maximal and minimum germination percent of seeds occurred 0 and 36 month after seed harvesting (Demirezen Yilmaz and Aksoy, 2007). *Rumex turcomanicus* Czerep. is one of green vegetable medicinally valuable plant. For many years this plant considered as a subspecies belongs to *R. tuberosus* L. species named *R. tuberosus* var. *turcomanicus* Rech., but in revision on the genus *Rumex* L. it was changed as a new species named *R. turcomanicus* Czerep. It was first reported by Czerepanov and accepted as a new species (The Plant List, 2010). It's a tuberose geophyte plant belongs to Irano-Turanian chorological type which distributed in Northeast of Iran especially on slopes of Binaloud mountains (Iran: Khorassan Province) (Ghahreman *et al.,* 2006), where its leaves are highly appreciated and consumed and is popularly known as "Sagh torshak". Despite the high consumption, its knowledge is inconsiderable and no attempt has been performed for its domestication. Previously, reported that dormancy isn't similar in different species of *Rumex* genus. The study conducted by Assche *et al.* (2002), on germination requirements, dormancy cycle and longevity of nine *Rumex* species, has shown that within one genus, rather striking differences were observed in germination ecology. They were stated that different species show the adaptations of the related species to their specific habitat (Assche *et al.,* 2002). Therefore, the aim of this study was to evaluate different seed harvesting times on seedling emergence and growth in *Rumex turcomanicus* Czerep.

Material and methods

Experimental site and materials

The seeds of *Rumex turcomanicus* Czerep. were collected from a natural site from Noghondar region around Mashhad -North east of Iran- (36°22' latitude and 59°17' longitude), in four times include 1; 2 weeks after fruiting (2WAF), 2; 6WAF, 3; 8WAF (mature seeds) and 4; 2 month after seed ripening (2MASR), which were divided in two groups. The seeds collected from dates of 1, 2, 3 and one group of 4 were sowed immediately after collecting and the second group of seeds from date of 4 (which were stored dry for 2 months at 25°C) were sowed 2 months later (4MASR). The experiment was conducted at agricultural college of Ferdowsi university of Mashhad, Iran in 2012-2013.

Emergence tests were carried out in compartmentalized nursery trays containing 50 (10 × 5) holes, each measuring 4 cm × 4 cm × 6 cm (depth), which were filled with 50% sterilized compost soil + 50% sterilized sand (V/V) under light/dark cycle conditions of 16/8 h at 23°C and 75% relative humidity placed in a glass greenhouse. Continuous watering was done to maintain the required moisture for germination.

Data Collection and statistical analysis

Seedlings were counted when the shoots emerged above the substratum surface. The number of emerged seedlings was recorded daily (seedling rate), and the number of final emerged seedlings (expressed in percentage) was counted after 50 days. The emergence rate was calculated according to Maguire's equation (Maguire, 1962): M = n1/t1 + n2/t2 + ... + n50/t50; where n1, n2, ..., n50 represent the number of emerged seeds at times t1 , t2 ,..., t50 (in days).

Ten seedlings were taken away randomly and seedling growth was measured by estimating seedling root length (SRL), seedling shoot length (SSL) and seedling length (SL), on the fiftieth day after emergence. In addition, fresh weights (SFW) and dry weights (SDW) of the sampled seedlings were taken. Dry weight for each plant was determined after drying the samples in an oven at 70°C for 48 hrs. Means of the 10 seedlings were used for the analyses of SRL, SRL, SL, SFW and SDW. The experimental design and statistical analyses were similar to those used for the germination test. Mean emergence time (MET) and seedling vigour index (SVI) were calculated using following formula.

Mean seedling emergence time (MET) calculated according the formula of Ellis and Roberts (1981):

$$MET = \sum \frac{\text{number of emerged seedlngs}}{\text{day of counting}}$$

Seedling vigour index (SVI) using the formula of Abdul- Baki and Anderson (1973):

$$SVI = \sum \frac{(\text{Seedling emergence percentage} \times \text{Seedling length(mm)}}{100}$$

Statistical analysis

The statistical analysis was performed using Microsoft Excel (2007) and JMP 8 software and means were compared using LSD test at $\alpha = 0.05$. The analysis of variance for percent emergence was performed on arcsine transformed data. Correlation coefficients (r^2) among different seedling traits were applied by SYSTAT13 software.

Results

According to the results, harvesting date was a significant difference on all of the studied traits ($p \leq 0.01$) (Tab.1).

Seedling emergence percentage (SEP) and seedling emergence rate (SER)

As it's shown in Fig. 1, the highest and lowest of SEP were obtained in the seeds which were sowed 2WAF (54.44%) and 4MASR (95.51%), respectively. Also, maximum and minimum of SER were observed 4MASR (15.32 seedling/day) and 2WAF (0.67 seedling/day), respectively (Tab. 2).

Tab. 1. Analysis of variance (mean of square) of seedling traits of *R. turcomanicus* Czerep, as affected by different seed harvesting times

Source of variance	df	SEP†	SER	MET	SVI	SFW	SDW	SRL	SSL	TSL	%FNS
Treatment	4	1154.07**††	158.72**	295.87**	10.42**	0.00538**	0.000024**	46.884**	10.346**	98.528**	1828.12**
Error	15	9.37	0.251	1.219	0.367	0.000376	3.539e-6	2.2929	0.6041	5.2480	13.87

Note: † SEP, SER, MET, SVI, SFW, SDW, SRL, SSL, TSL and %FNS are indicated seedling emergence percentage, seedling emergence rate, mean emergence time, seedling vigour index, seedling fresh weight, seedling dry weight, seedling root and shoot length, total seedling length and %final normal seedling, respectively

†† ns, *, ** and *** are non-significant and significant at 5, 1 and 0.1% probability levels, respectively

Tab. 2. Mean comparison of seedling traits of *R. turcomanicus* Czerep, as affected by different seed harvesting times

Seed harvesting dates	SER†	SFW (g)	SDW (g)	SRL (mm)	SSL (mm)	TSL (mm)
2WAF††	0.67d†††	0.0536c	0.00422d	18.27c	10.13d	28.40d
6WAF	0.953d	0.0623c	0.00575cd	19.67bc	9.82cd	29.49cd
8WAF	3.37c	0.0709c	0.00724bc	21.53b	11.27bc	32.80c
2MASR	9.35b	0.1028b	0.00926ab	24.61a	11.77b	36.38b
4MASR	15.32a	0.1431a	0.01017a	26.54a	13.87a	40.40a

Note: † SER, SFW, SDW, SRL, SSL and TSL are indicated seedling emergence rate, seedling fresh weight, seedling dry weight, seedling root and shoot length and total seedling length, respectively;

†† 2WAF, 6WAF, 8WAF, 2MASR and 4MASR are indicate seed harvesting times 2 weeks after fruiting, 4 weeks after fruiting, 8 weeks after fruiting, 2 months after seed ripening and 4 months after seed ripening, respectively;

††† Similar letters in each column show non-significant differences according to LSD Test at 5% level of probability

Mean emergence time (MET) and seedling vigour index (SVI)

The highest and lowest of MET were obtained 20.90 and 1.95 days, when the seeds were sowed 2WAF and 4MASR, respectively (Fig. 2). The highest and lowest of SVI were obtained in the seeds which were sowed 4MASR (6.74) and 2WAF (2.82), respectively (Fig. 3).

Seedling fresh (SFW) and dry weight (SDW)

The highest and lowest of SFW and SDW were observed in the seeds which were sowed 4MASR (0.1431 and 0.01017 g) and 2WAF (0.0536 and 0.00422 g), respectively (Tab. 2).

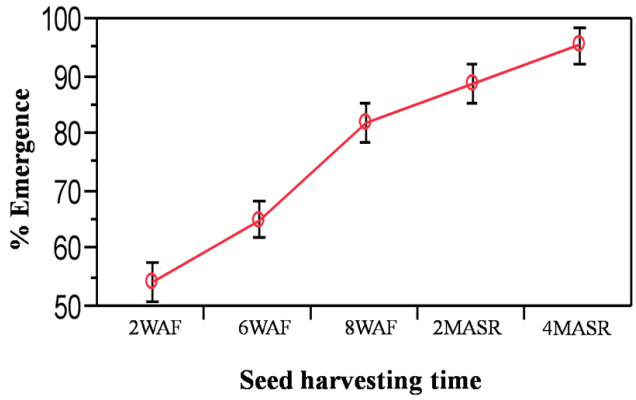

Fig. 1. Effect of different seed harvesting times on mean seedling emergence percentage of *R. turcomanicus* Czerep.

Note: 2WAF, 6WAF, 8WAF, 2MASR and 4MASR are indicate seed harvesting times 2 weeks after fruiting, 4 weeks after fruiting, 8 weeks after fruiting, 2 months after seed ripening and 4 months after seed ripening, respectively

Seedling root (SRL), soot length (SSL) and total seedling length (TSL)

The highest of SRL, SSL and TSL were measured in seed sowing dates at 4MASR, 26.54, 13.87 and 40.40 mm, respectively, and the lowest of these traits were obtained 18.27, 10.13 and 28.40, respectively, when the seeds were sowed 2WAF (Tab. 2).

% Final normal seedling (%FNS)

Maximum (85.29) and minimum (38.87) of % FNS was obtained in seed sowing dates at 4MASR and 2WAF, respectively (Fig. 4).

Relationship among seedling traits:

Correlation coefficients (r^2) among different seedling traits of the crop are listed in Tab. 3. The r^2 values of pairs

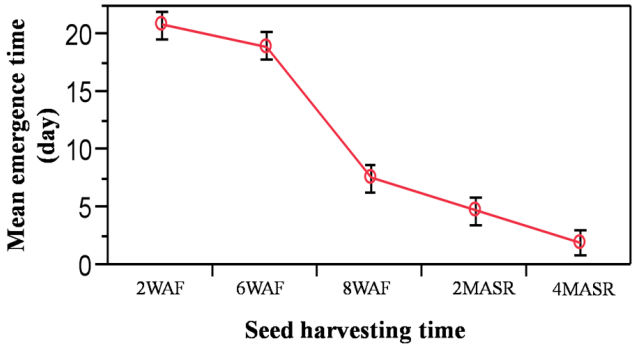

Fig. 2. Effect of different seed harvesting times on mean emergence time in *R. turcomanicus* Czerep.

Note: 2WAF, 6WAF, 8WAF, 2MASR and 4MASR are indicate seed harvesting times 2 weeks after fruiting, 4 weeks after fruiting, 8 weeks after fruiting, 2 months after seed ripening and 4 months after seed ripening, respectively

Tab. 3. Pearson correlation coefficients (n=20) seedling vigour traits of *R. turcomanicus* Czerep collected in different times

Trait	SEP†	SER	MET	SVI	SFW	SDW	SRL	SSL	TSL
SER	0.87**††								
MET	-0.961**	-0.869**							
SVI	0.926**	0.905**	-0.895**						
SFW	0.781**	0.894**	-0.765**	0.724**					
SDW	0.752**	0.737**	-0.777**	0.648**	0.829**				
SRL	0.825**	0.897**	-0.875**	0.792**	0.857**	0.817**			
SSL	0.739**	0.881**	-0.818**	0.74**	0.847**	0.743**	0.947**		
TSL	0.806**	0.902**	-0.867**	0.785**	0.864**	0.802**	0.995**	0.976**	
%FNS	0.962**	0.923v	-0.954**	0.929**	0.817**	0.759**	0.869**	0.793**	0.855**

Note: †SEP, SER, MET, SVI, SFW, SDW, SRL, SSL, TSL and %FNS are indicated seedling emergence percentage, seedling emergence rate, mean emergence time, seedling vigour index, seedling fresh weight, seedling dry weight, seedling root and shoot length, total seedling length and %final normal seedling, respectively

†† *, ** Significant at 5% and 1%, respectively

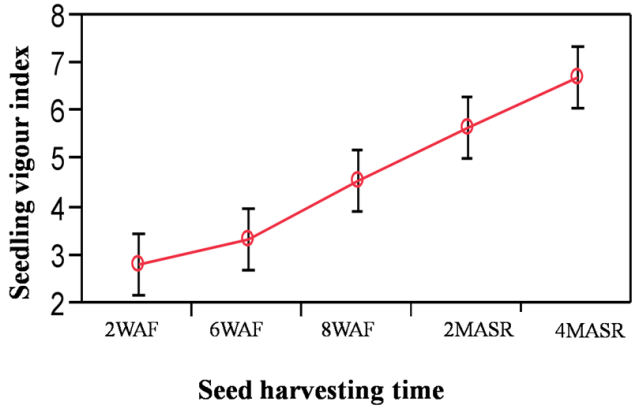

Fig. 3. Effect of different seed harvesting times on mean seedling vigour index in *R. turcomanicus* Czerep.

Note: 2WAF, 6WAF, 8WAF, 2MASR and 4MASR are indicate seed harvesting times 2 weeks after fruiting, 4 weeks after fruiting, 8 weeks after fruiting, 2 months after seed ripening and 4 months after seed ripening, respectively

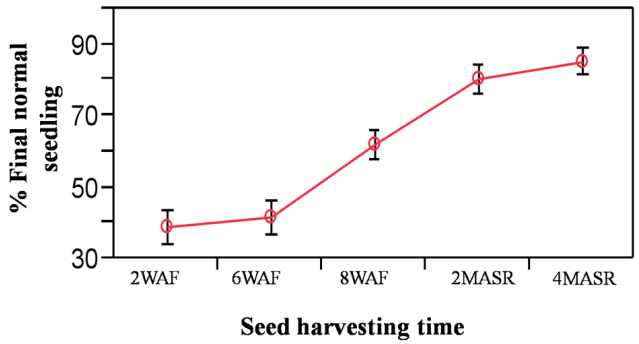

Fig. 4. Effect of different seed harvesting times on mean %final normal seedling in *R. turcomanicus* Czerep.

Note: 2WAF, 6WAF, 8WAF, 2MASR and 4MASR are indicate seed harvesting times 2 weeks after fruiting, 4 weeks after fruiting, 8 weeks after fruiting, 2 months after seed ripening and 4 months after seed ripening, respectively

of all traits were significant ($p \leq 0.01$). The association between emergence percentage and other traits was negative. Relationship between %final normal seedling and mean emergence dates and between %final normal seedling and emergence percentage was significantly negative and positive, respectively (Tab. 3).

Discussion

As the results showed, with increasing of seed maturity on mother plant, germination and subsequently seedling emergence as well as other studied characteristics were improved, significantly. The results of this experiment are in agreement with those obtained by Povilaitis (1956), who stated that fresh seed of *R. acetosella* germinated very poorly but germination improved slowly in dry storage. Unlike, the results obtained by Yilmaz and Aksoy (2007), on seed germination of *R. scutatus*, this experiment showed a positive relationship between germination rate and seed age of *R. turcomanicus*.

It seems to be a kind of dormancy in immature seeds that prevent from proper seedling emergence in fresh seeds. In *R. crispus*, which is considered one of the most troublesome weeds, seedlings can emerge throughout the growing season and seeds that remain on the parent plant over winter posses a certain level of dormancy, leading to later and more intermittent emergence compared to the seeds dispersed in autumn (Pye and Andersson, 2008). Trials were carried out to investigate the effects of light and temperature on germination of *R. obtusifolius*. After several months of storage, seeds gradually lost dormancy and became photosensitive. (Benvenuti *et al.,* 2001). According to Asrar (2011), embryos of *R. vesicaricus* seeds have deep physiological dormancy and with 200 ppm gibberellic acid (GA_3) for 48 hrs at 20°C germination promoted through break the dormancy and improving some chemical characteristics of *R. vesicaricus* seeds. Conversely, Bewley and Black (1994) were reported that embryos of *R. vesicaricus* seeds are morpholically immature and thus require a period of further development before they are able to germinate. Such a morphological dormancy may be in the immature seeds (collected 2WAF) of *R. turcomanicus*, which is broken with increasing of seed maturity.

Conclusions

Based on the results of this experiment, it seems that the problem to germination in most of the *rumex turco-*

manicus Czerep. seeds is probably due to a kind of morphological dormancy, which is remained in most of the fresh seeds, which were collected 2WAF, and eliminated in the mature seeds, which were collected 2MASR. Also dry seed storage of the mature seeds for two months was improved seedling emergence and vigour, significantly.

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