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EFFECT OF PRE-CHILLING AND TUBER SIZE ON LESSER CELANDINE (*RANUNCULUS FICARIA*) GERMINATION OF TUBEROUS ROOT

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ABSTRACT
In order to study the effect of pre-chilling and tuber size on germination of *Ranunculus ficaria* tubers, two separate experiments were conducted in 2008. The first experiment was a completely randomized design with factorial arrangement and four replications. The factors included storage time in incubator and storage temperature. In the second experiment, to evaluate the effect of tuber size, tubers were divided into six groups: very small, small, medium, fingerlike, tall and broken tubers. The results showed that the highest percentage of germination occurred in tubers which had been stored more than 2 weeks at 4 or 8 ˚C. No significant differences were observed neither between 4 and 8˚C storage temperature, nor between the different durations of storage. Germination of the fingerlike group and small tubers was the highest (95%), however, very small, small and broken tubers had the lowest germination percentage.

Key words: pre-chilling, tuber size, germination, *Ranunculus ficaria*

RÉSUMÉ
Afin d'étudier l'effet du pré-refroidissement et de taille des tubercules sur la germination de tubercules de *Ranunculus ficaria* deux expériences ont été menées en 2008. La première expérience a été un dispositif complètement randomisé avec un arrangement factoriel et quatre repetitions. Les facteurs comprenaient le temps de stockage et la température d'incubation. Dans la deuxième expérience, pour évaluer l'effet de la taille des tubercules, les tubercules ont été divisés en six groupes: très petits, petits, moyens, digités, grands et brisures de tubercules. Les résultats ont montré que le pourcentage le plus élevé de germination a eu lieu pour les tubercules qui ont été entreposés plus de 2 semaines à 4 ou 8 ˚ C**.** Aucune différence significative n'a été observée ni entre 4 et 8 ˚ C température de stockage, ni entre les différentes durées de stockage. La germination du groupe digité et des petits tubercules a été la plus élevée (95%), Toutefois, les très petits, petits et tubercules cassés ont eu le plus faible pourcentage de germination.

Mots-clés : pré-réfrigération, taille des tubercules, germination,*Ranunculus Ficaria*

INTRODUCTION

Lesser celandine, also known as fig buttercup, is a herbaceous, perennial plant of the *Ranunculaceae* family. It has shiny dark green and kidney-to-heart- -shaped stalked leaves. The flowers open in March and April, they have eight glossy, butter-yellow petals, and are located singly on long delicate stalks that rise above the leaves. Lesser celandine is an unusual dicotyledon because it has seedlings with a single cotyledon.

This weed has fibrous roots and numerous root tubers, (CLAPHAM *et al*. 1987). The first basal tubers develop as adventitious roots on the axillary buds of the basal leaves, (TAYLOR & MARKHAM 1978). Later subterranean tubers are formed by the appearance of new root initials and buds on the existing tubers. The tubers enter a 6-month resting phase at the end of May. After the aerial parts of the plant have died down, a distinct white bud develops and gradually enlarges on the newly formed group of root tubers. Other smaller buds may also develop. Tuber dormancy is broken by chilling, (GRIM *et al.* 1988). Maintaining tubers of *Ranunculus ficaria* ssp. *bulbifera* at 15-20˚C prolonged the resting phase indefinitely, (TAYLOR & MARKHAM 1978).

The resting buds on the root tubers turn green and begin to elongate about December and a number of adventitious roots begin to develop. The foliage leaves of the basal rosette begin to unfold in January, (GRIM *et al.* 1988). A further series of roots develop that will become the new tubers. The bulbils or tubercles that develop on the leaf axils are similar to the basal tubers, (TAYLOR & MARKHAM 1978). They are formed from an adventitious root initial, the root swells and the bud remains near the point of attachment to the plant. The first few leaf initials may develop as scale leaves, (BOND et al. 2007).

Fragmentation of the groups of basal tubers into individual units, each with a single bud, is an efficient means of vegetative propagation, (TAYLOR & MARKHAM 1978). Both native forms can spread from the root tubers following ploughing or digging. In addition, in subspecies *bulbifera,* the leaf bulbils separate off as the leaves die down. It can develop up to 24 bulbils per plant. Subspecies *bulbifera* is more considered as the weedy form by SALISBURY (1962). However, according to (GRIME *et al.* 1988), this subspecies would rather be a woodland plant and *Ranunculus ficaria* ssp. *ficaria* is mainly found in open and disturbed habitats and gardens. Lesser celandine is an exotic spring ephemeral and a vigorous growing groundcover that forms large, dense patches on the forest floor, displacing and preventing native plants from co-occurring. In both woodland and open habitats, early growth takes place in short days when temperatures and light levels are low. The primary means of reproduction in this species is through production and dispersal of the subterranean bulblets or tubers.

The lesser celandine has become increasingly invasive in western Iran, especially in the wheat fields. Increased knowledge of lesser celandine tuber germination biology would facilitate the development of an optimum control programs. The object of research was to determine the effect of pre-chilling and tuber size of lesser celandine tuber germination.

MATERIAL AND METHODS

Tubers were collected from a wheat field located to the South West of Khoramabad, Iran in May 2008. This study was conducted at the Weed Science Laboratory of the Faculty of Agriculture, Ferdowsi University of Mashhad.

The tubers of lesser celandine were sterilized for 4 min. in a 1% sodium hypochlorite solution. Then, the collected tubers were divided into two groups and placed immediately in germinators at 10 and 15˚C, for two weeks. The lack of germination at both temperatures indicated that all buds were dormant. Therefore, pre-chilling experiments were conducted in order to break the dormancy of tubers.

Pre-chilling experiments were conducted in a completely randomized design with factorial arrangement of treatments and four replications. The factors included time of storage in refrigerator (7, 14, 21 and 28 days) and storage temperature (2, 4 and 8˚C). Five Tubers were placed on top of moist filter paper in each Petri dish. Each Petri dish was considered as a replication. To pre-chilling, the Petri dishes were placed in an incubator. When the periods of pre-chilling were completed, the tubers were placed in a germinator for 20 days at 14˚C with 60% moisture and darkness.

To evaluate the effect of tuber size on germination, another experiment was conducted in a completely randomized design with four replications. The tubers were divided into six size groups - very small (<0.02g), small (0.03-0.04g), medium (0.06-0.12g), fingerlike (0.15-0.64g), tall (0.1-0.27g) and broken (0.07-0.18g) - and each one was considered as an experiment treatment. Five tubers of each group were placed on moist filter paper in each Petri dish (with 5 ml distilled water). The Petri dishes were placed in a germinator for 20 days at 14˚C. Every other day, the Petri dishes were checked and the tubers with visible white buds, were considered as germinated tubers. On this basis germination percentage and germination rate (no. of germinated tubers per day) was calculated.

All data were analyzed using Minitab ver. 13 and mean differences (at 5% level) were calculated using LSD test.

RESULTS

The results showed that time and temperature had significant effect on tuber germination (Table I).

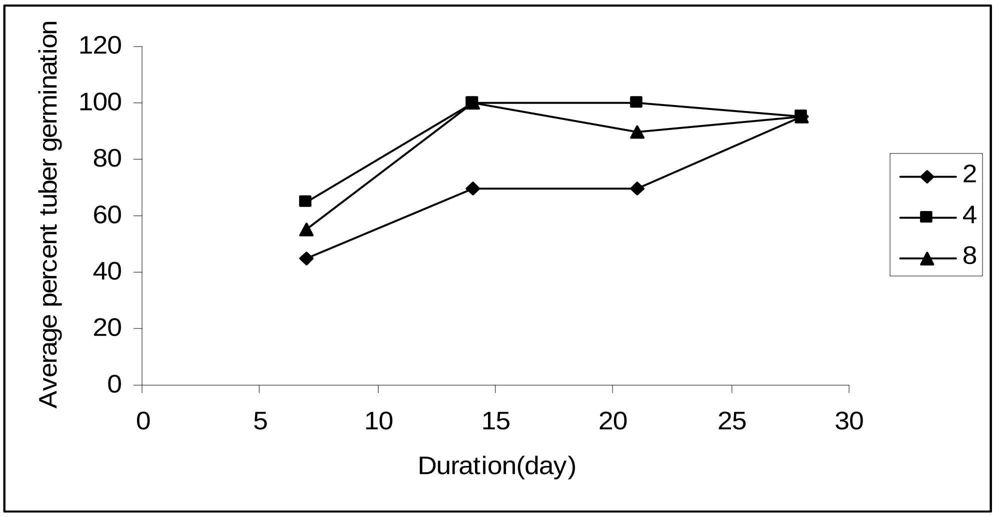
The germination rate of the two-week pre-chilled tubers was significantly higher than that of the one-week pre-chilled tubers (Fig1). Germination of pre-chilled tubers at 4 and 8˚C for 14 days was about 100%, compared with non pre-chilled tubers with 0% germination. No significant differences were observed between 2 and 4 weeks pre-chilling on the germination of the tubers. A pre-chilling treatment of 2℃ for 7 days was redu ced tuber germination (40%). Germination rate was the highest for 4˚C on 28th day (Fig2).

Table I- Analysis of variance for the effect of pre-chilling on germination of *R. ficaria* (Analyse de la variance de l'effet de pré-refroidissement sur la germination de *R.ficaria*)

Source of variation	Df	Ms
Week	3	3933.3 *
Temperature	2	1733.3 *
Temperature*week	6	266.7 ns
Error	36	194.4
Total	47	

ns: not significant, *: significant at 0.05

Figure 1: Effect of pre-chilling duration and temperature on germination of *R. ficaria* (Effet de la durée de pré-refroidissement et de la température sur la germination de *R.ficaria*) (LSD=0/98)



The results also indicated that the size of tubers had significant effect on germination (Table II). The fingerlike group and small tubers had the highest percentage of germination, while the medium, broken, very small and tall tubers showed 70%, 50%, 45% and 35 % germination, respectively. However, no significant difference was observed among broken, very small and tall tubers. During the study, only the tall tubers were highly affected by root rot (45%). Germination rate was the highest in the fingerlike group and small tubers (Fig 4). By the end of the experiment, the fingerlike group produced about 60% fibrous roots but other group tubers had only few roots (about 10%).

Figure2: Effect of pre-chilling duration on Germination rate of tubers *R. ficaria* (Effet de la durée de pré-refroidissement sur le taux de germination des tubercules *R.ficaria*)

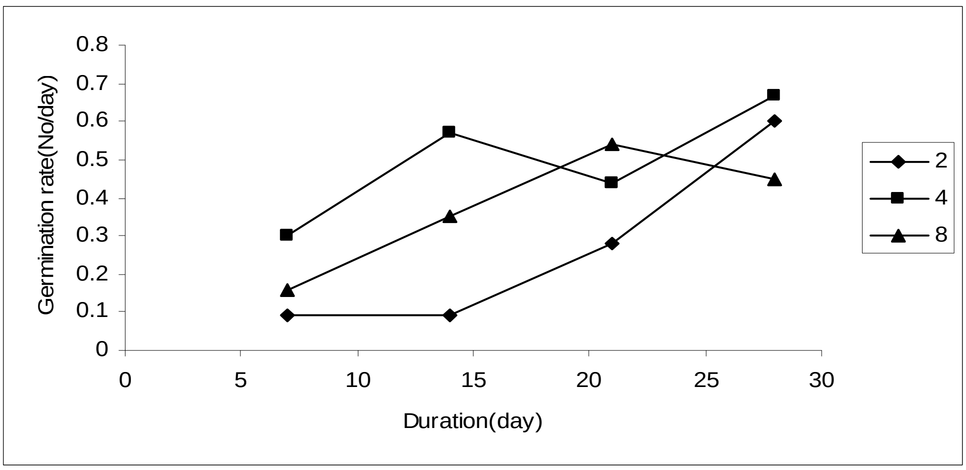


Table II- Analysis of variance effect of the tuber form on germination of *R. ficaria* (Analyse de la variance de l'effet sur la germination des tubercules de *R.ficaria*)

Source of Variation	DF	MS
Replication	3	133.3 ns
Form tuber	5	2986.7 *
Error	15	186.7
Total	23	

ns: not significant, *: significant at 0.05

Figure 3: Effect of the tuber form on the germination of *R.ficaria* (LSd=0/89) (Effet de la forme des tubercules sur la germination des *R.ficaria*)

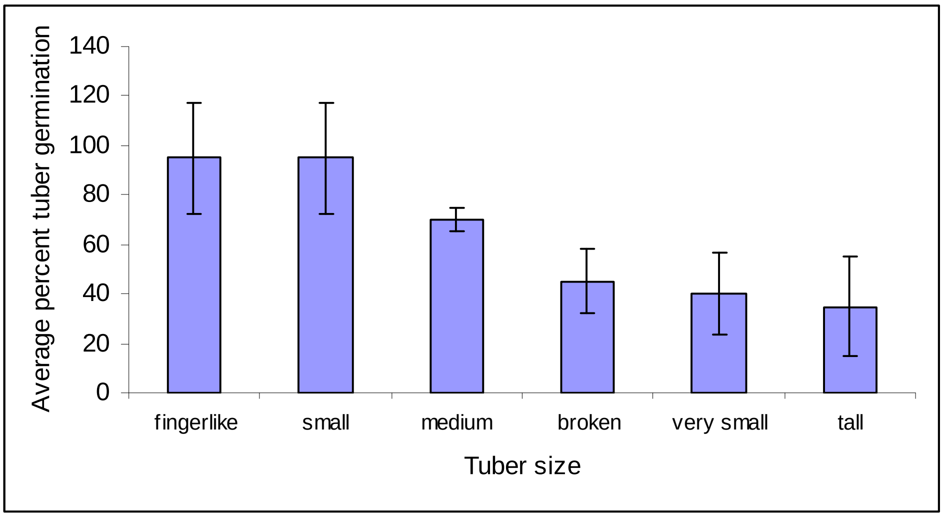
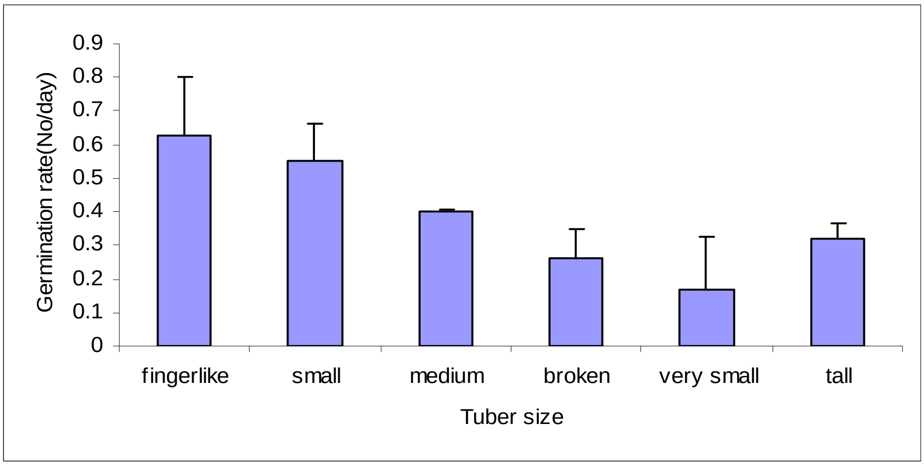


Figure4: Germination rate of six tuber forms of *R.ficaria* (Taux de germination des tubercules de six formes de *R.ficaria*)



DISCUSSION

According to the results, the dormancy of newly produced lesser celandine tuber could be broken by low temperature.

Germination percentages were relatively high at 4˚C and 8˚C for most of the 2 weeks. Tuber dormancy is broken by chilling, (GRIME *et al.* 1988). Maintaining tubers of ssp. *bulbifera* at 15-20˚C prolong the resting phase indefinitely, (TAYLOR & MARKHAM 1978). Lesser celandine is often the most difficult to control. The storage organ often remains dormant for many months therefore surviving fallows. The length of the rest period tubers of *R.ficaria* is dependent on the temperature, but is not affected either by daylength or the level of irradiance, (MARKHAM, 1970).

Tuberous root of *Ranunculus asiaticus* L. require exposure to temperature averaging 4–5 °C for 4–5 wk or 2 ℃ for 2 wk Cold treatment applied prior to planting for break dormancy and start growth. Also tuber *of Anemone coronaria* L. could germination after exposure to 2–10 ℃ for 4–6 wk interactions with LD, high temperatures, and drought stress. Geophytic species respond to many environmental signals that determine when to enter or exit dormancy, including temperature, moisture, and photoperiod. For example, with Dahlia hybrids (*D. coccinea* Cav. x *D. pinnata* Cav.) tuberous root formation is induced by photoperiods of 11–12 h or less. The dormant tuberous roots do not immediately resume growth unless they have been exposed to 0 to 10 ℃ for 6 weeks, (KONISHI & INABA, 1967; MOSER & HESS 1968). Geophytes are found in a range of climates from tropical to arctic and, therefore, differ greatly in response to temperature. Species such as Tulipa require exposure to temperatures averaging 5 ℃ fo at least 10 to 12 weeks to break dormancy, (LE NARD & DE HERTOGH, 1993a).

In the *Ranunculus asiaticus*, cold treatment of bulbs at 5℃ for 4 weeks initia ted flower primordia (sepal) in all plants 25 days after the planting. But the sprouting of 0℃ stored bulbs was delayed 60 days compared to 20℃ stored bulbs, (OHKAWA, 198 6).

The percentage of final germination in the fingerlike group and small tubers were increased. The percentage of smaller tuber size was reduced due to decreased availability of storage tissue to support germination of tubers. Lower germination rate in the tall tubers could be due to high respiration rate and susceptibility of these type of tubers to disease.

The storage is especially important in perennial plants occurring in regions with cold winters. Spring growth of these plants strongly depends on the reserves accumulated during the previous season, (SHAVER *et al*. 1976; KLIMES *et al*. 1993; KUB´IN & MELZER, 1996).

Like for other perennial geophytes, the size and age of the bulbs are determining factors for the ability of reproductive development, (RUITERS *et al*. 1993).

Plants must attain proper photosynthetic storage capacity before being subjected to reproductive conditions. In addition, early exposure to reproductive conditions before storage organs reach sufficient size (end of juvenility) may decrease uniformity of flowering, (CAMERON *et al*. 1996). It has been determined experimentally that the size of tubers of *R.ficaria* is also an important factor in determining the length of the rest period; within the range of tuber sizes in field population, the larger tubers develop more rapidly than the smaller ones when exposed to low temperature(5℃). The fragmentation of basal-gr oup, such that each unit has at least one bud, thus possessing the potential for new growth, is very efficient means of vegetative propagation,(MARKHAM,1970). In all populations of R.ficaria, plant size is an important factor in flower production: the larger the initial tuber weight the greater the tendency to flower, (TAYLOR & MARKHAM 1978).

CONCLUSION

Lesser celandine grows well in cold environments. And its growth starts, after pre-chilling for 4 weeks at 4℃. The germination of fingerlike tubers was the highest thanks to greater reserves which could allow a potential rapid colonization of sites. So in environments that are open and colonizable, the tubers will be at an advantage. The large heavier tubers of *R.ficaria* should have large food reserves, which makes it possible for the seedlings to emerge as a more completely developed plantlet, to survive longer and grow to a more aggressive size in an environment that is deprived of resources. The small tubers which have the highest germination rate can be removed easily and rapidly to a far distance.

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