PL ISSN 1429-3862
E-ISSN 2083-599X

Plant Breeding and Seed Science

80
Volume

PLANT BREEDING AND ACCLIMATIZATION INSTITUTE- NATIONAL RESEARCH INSTITUTE
RADZIKÓW 2019

PL ISSN 1429-3862
E-ISSN: 2083-599X

Plant Breeding and Seed Science

80

Volume

PLANT BREEDING AND ACCLIMATIZATION INSTITUTE—NATIONAL RESEARCH INSTITUTE
RADZIKÓW 2019

Founded in 1957 as „Hodowla Roślin Aklimatyzacja i Nasiennictwo” in Plant Breeding and Acclimatization Institute (since 2010 Plant Breeding and Acclimatization Institute — National Research Institute). Edited by Jadwiga Lekczyńska (1957—1982), Stanisław Starzycki (1983), Henryk J. Czembor (1984—1999) and since 2000 by Edward Arseniuk).

Starting from Volume 40 (in 1996) published in English as Plant Breeding and Seed Science.

Since 2005 our Journal is citted in Thomson Master Journal List. Since 2009 the version online is on the Metapress Platform by Versita Ltd.

Digital version is also available on our website.

EDITORIAL OFFICE
Plant Breeding and Acclimatization Institute— National Research Institute,
Department of Scientific Information and Editorial Board, Radzików,
05-870 Błonie, Poland, Phone: +48 (22) 733 45 95, Fax: +48 (22) 725 47 14,
e-mail: pb&ss@ihar.edu.pl

Managing Editor: Marek Czuba, e-mail: m.czuba@ihar.edu.pl

PLANT BREEDING AND SEED SCIENCE
Volume 80 2019

DOI: 10.37317/ pbss-2019-0008

Anna Podlewska-Przetakiewicz *[1]; Dorota Milczarek [2]

[1] Plant Breeding and Acclimatization Institute-National Breeding Institute, Research Centre Radzikow, Department of Plant Pathology, Radzikow, 05-870 Blonie, Poland;
[2] PlantBreeding and Acclimatization Institute – National Research Institute, Research Centre Młochów, Platanowa 19, 05-831 Młochów, Poland
* Corresponding author e-mail: a.przetakiewicz@ihar.edu.pl

MOLECULAR METHODS OF CHARACTERIZATION AND IDENTIFICATION OF *GLOBODERA ROSTOCHIENSIS* AND *GLOBODERA PALLIDA* POPULATIONS

ABSTRACT

The cyst nematodes belonging to the genus *Globodera* are big worldwide problem in countries were *Solanaceaous* plants growing. Knowledge of species-composition in populations of *Globodera rostochiensis* and *Globodera pallida* is very important for selection of appropriate measure of nematode regulations occurrence. Inter- and intraspecific variability among species of *Globodera rostochiensis* and *Globodera pallida* were studied intensively with the use of molecular analyses of DNA methods. This review summarize and compare of methods chosen to distinguishing between *Globodera*, both pathotypes and species.

Key words: *Globodera*; inter-and intraspecies identification; molecular methods; potato cyst nematode

INTRODUCTION

Globodera rostochiensis (Wollenweber) Behrens and *G. pallida* (Stone) Behrens belongs to the most economically important pests of potato causing up to 50% of yield damages (Nicol *et al.*, 2011). They were introduced into Europe from South America in the 1600s (Evans *et al.*, 1975; Baldwin and Mundo-Ocampo, 1991) and knowledge of the genetic characteristics of each separate introduction might form the basis for classifying the various populations and virulence groups. Pathotype scheme proposed by Kort in 1977 differentiates some pathotypes on the basis of qualitative differences in relation to major resistance genes and others on the basis of quantitative differences in reproduction of populations on differential hosts with polygenic resistance (Kort *et al.*, 1977).

Communicated by Grzegorz Żurek

Nowadays, many molecular techniques based on DNA analysis is used to searching for variations among nematode populations comes from different geographical regions. Molecular data could be a powerful source of information for the systematics of nematodes and conduct to the determine the range of genetic diversity in relation to virulence of potato cyst nematode (PCN) populations (Hyman and Powers, 1991; Ferris *et al.*, 1991; Baldwin, 1992; Ferris and Ferris, 1992; Bossis and Mugniery, 1993; Powers and Adams, 1993; Blok *et al.*, 1998). Reliable identification and characterization of nematode populations is a prerequisite to studying their genetic variation, interaction with host plants, virulence and to many aspects of PCN control and management. The choice of molecular technique used to the estimation of genetic diversity of populations depends on research question.

MOLECULAR TECHNIQUES OF PCN CHARACTERIZATION

rDNA

Region of rDNA in eukaryotic species consists three ribosomal RNA genes - 18S, 28S, 5.8, internal transcribed regions – ITS 1 and ITS2 and an external non -transcribed spacer region. In nematodes three ribosomal genes are the best characterized gene regions (Powers, 2004). They are highly conserve but the comparative analysis of differences in coding and non-coding regions of ribosomal DNA (rDNA) can be a popular method for inter- and intraspecies identification of many organisms. DNA sequencing of ITS regions within rDNA allows revealing single base-pair substitution and analysis of this regions has been used to examine phylogenetic relationships between population of the same species. Phylogenetic analysis of 41 ITS region sequences of *Globodera* parasitizing solanaceous plants obtained by Subbotin and co-authors (2000) suggest that the rDNA in genome of *G. rostochiensis* and *G. pallida* populations is present as a mixture of haplotypes with different sequences. Ferris *et al.*, (1993) compared sequence data from internal transcribed spacer (ITS) of the *Globodera* spp. and found that they have characteristic inter- and intraspecific variations and that the choice of region in the rDNA can influence the amount of variability detected between isolates.

In 1998 Blok studied intraspecific variation of 18 population of *G. pallida* from Europe and South America in relation to their original introduction into Europe amplifying the repeated region of ITS1/5,8/ITS2 together with 3' end of 18S and 5' end of 28S gene. They found one population of *G. pallida* from South America more distinct then the others, in different molecular method: RFLP and sequence analysis (Blok *et al.*, 1998) and earlier in SSR and RAPD method (Blok *et al.*, 1997) in comparison of ITS2 region of all tested populations. Identical approaches carry out on 16 Ukrainian population of *G. pallida* and *G. rostochiensis* by Pypylenko *et al.* (2008) shows similarity of *Globodera pallida* population in 97,8% based on sequences 938bp in comparison to published sequences of European population of PCN described by Blok *et al.* (1998) and Subbotin *et al.* (2000). Polymorphism of sequence of the first inter-

nal transcribed spacer (ITS1) has been widely used to identifying and assessing genetic variability of three Russian population of *Globodera rostochiensis* comes from different geographical localities (Chrisanfova *et al.*, 2008). Low variation of the ITS-1 sequences and high conservativeness of this genome region failed to distinguish the isolates groups within the species with the used of this particular method. Comparative analysis of rDNA (fragments ITS1/5.8S/ ITS2) sequences of 16 polish *Globodera* populations show 97-98% identify in *G. rostochiensis* and 94-95% identity in *G. pallida* isolates and allow for splitting populations into three phylogenetic groups originate from Central and Eastern part of the country (Nowaczyk *et al.*, 2011). Molecular characterization of Serbian *Globodera* isolates reported by Oro *et al.* (2012) based on comparison of variation occurred in ITS-1 region assumed that possible ancestors of PCN population originated from Peru but presence of *G. pallida* is not the result of direct import of infected potatoes from Latin America but from England where the population from York has the same sequence like Serbian ones.

mtDNA

Animal mitochondrial genomes (mtDNA) are often used in phylogenetic and population studies. Due to maternal mode of inheritance and lack of recombination mtDNA seems to be the best marker for searching of evolutionary connection between populations (Avise, 1994; Harrison, 1989; Birky, 2008). At present there are available a complete nucleotide sequences for 76 species which revealed differences in size, structure and gene content (Armstrong *et al.*, 2000). The mtDNA encodes the 22 transfer RNAs, two ribosomal RNAs and 12 or 13 proteins involved in transport of electrons and oxidative phosphorylation (Pont-Kingdon *et al.*, 1995). In metazoan mtDNA can occur as a single circular molecules, a linear chromosome or two 8-kb linear molecules (Bridge *et al.*, 1992).

In 2000 Armstrong provided evidence about multipartite structure of mtDNA of *Globodera pallida*. Molecular approaches carry out on British population of *G. pallida* reveal at least 6 small, circular mitochondrial DNAs (scmtDNAs I - VI) differ in size. These scmtDNA contained mitochondrial gene coding sequences and about 2kb non-coding region common to all small circular mitochondrial DNAs (Armstrong *et al.*, 2000). The authors suggested that scmtDNAs are present in all *G. pallida* but their frequencies vary between different populations (Armstrong *et al.*, 2007). For example, while scmtDNAs I and III were not detected in several UK populations, scmtDNA IV was present in all population analyzed. Last study shows that scmtDNA IV seems to be the most evolutionary stable than other subgenomic circles and may be used for studying genetic interactions between populations. Moreover, only scmtDNA IV contains rRNA genes which are necessary for the translation of mitochondrial protein. The scmtDNA IV region can decise populations as monophyletic what indicates this DNA region may be a useful marker for *G. pallida*. Analysis of scmtDNA sequences variation can be a powerful tool to research relationship between European *G. pallida* populations and their South American ancestors (Armstrong *et al.*, 2007).

On base of maternal mode of mtDNA inheritance and theory that recombinant products are indistinguishable from their progenitor molecules, Hoolahan in 2012 investigated past and contemporary recombination of British population of *G. pallida*. Past recombination was detected and confirmed between a South American and several UK population of white potato cyst nematode. In their study, progeny from experimental crosses of tested population of *G. pallida* had no evidence of contemporary recombination between the mtDNA of the maternal and paternal populations (Hoolahan *et al.*, 2012) what support current arguments that animal mtDNA recombination events are rare (Kivisild *et al.*, 2000; Innan and Nordberg, 2002).

RFLP

Molecular analysis based on restriction digest of total DNA (Restriction Fragment Length Polymorphisms - RFLP) are being used to observe genetic variation both between and within species. Investigations of variability within population are based on a sequence information from different populations and on using various clones. Inter- and intraspecific polymorphism between reference populations of *G. rostochiensis* and *G. pallida* has been observed for the first time by Burrows and Boffey in 1986. This method was used several times to examine genetic variation between different population of *G. pallida* but the results did not correlate with a type of pathotype (De Jong *et al*., 1989; Schnick *et al*., 1990; Phillips *et al*., 1992).

The studies of rDNA using PCR-RFLP method are attractive in case of small organisms like nematodes. In 1998 Blok reported variation in the multiple copies of rDNA of eighteen populations of *G. pallida* from Europe and South America. Authors conducting RFLP analysis of PCR products of ribosomal cistron out of six digestion enzymes found one that discriminated among most of the tested populations. The results support already existing studies that the majority of European population of *G. pallida* derived from one source with few exceptions (Blok *et al*., 1998).

RFPLs analysis of ITS-PCR products were carry out by Subbotin in 2000 on group of Russian populations of *Globodera rostochiensis* and the other *Globodera* species (Subbotin *et al*., 2000). Researchers used RFLP catalogue and sequence information from different populations or species and set of digestion enzymes to compare PCR products of nematode populations. Results of work revealed that rDNA in the genomes of *G. rostochiensis* populations is present as a mixture of haplotypes with different sequences and RFLP profiles and sequences of ITS region can be used rather for inter- than for intra-specific identification of *Globodera* species.

RAPD

Random Amplified Polymorphic DNA (RAPD) is one of the high sensitive PCR-based technique which involves the use of single 8-12 nucleotide length primers to amplification of many discrete and random DNA segments in the target genome (Welsh and McClelland, 1990). Genetic relationships between Dutch populations of *G. rostochiensis* and *G. pallida* analyzed by Folkertsma *et*

al. (1994) with the use of RAPDs has shown pathotypes designation within the first species but not with the second. They found that isolates of *G. rostochiensis* classified as one pathotype were distinguishable by a number of unique RAPD fragments. Intra-species differences in European and South American populations of *G. pallida* and two populations of *G. rostochiensis* was examined by Blok *et al.* (1997). In their study both populations of golden PCN, identificated as Ro1, were similar but they reported higher dissimilarity of white PCN isolates then in a Dutch surveys. Research carried out by Bendezu *et al.* in 1998 on nine populations of *G. rostochiensis* from UK, Bolivia, the Netherlands and Germany with the use of four primers shown genomic similarity among European populations of yellow potato cyst nematode in 82% and among UK populations in 89%. Intra-species differences in virulence were investigated by Pastrik *et al.* (1995) on German *G. pallida* populations and the partially resistant potato cultivar Darwina. RAPD patterns with the use of 40 primers showed generally high similarity of selected and unselected for virulence PCN populations except of two non-homologous DNA fragments present in selected ones. Pastrik interpreted that one of this DNA fragment indicates a correlation with the particular type of virulence in individual population (Pastrik *et al.*, 1995).

RAPD technique was used by Greiner *et al.* in 2001 to reveal a possible phylogeny between populations comes from different continents and determine the origin of infestation. Authors compare Peruvian and European populations of *G. pallida* and the results showed clear intra-specific distinction between two tested groups of population. In contrary to their results Hlaoua *et al.* (2008) studied similarity of Tunisian and European populations of white potato cyst nematode and proved high similarity of both clade of populations.

Conceição *et al.* (2003) tested genetic variability of 32 populations of *Globodera rostochiensis* and three of *G. pallida* from different regions of Portugal. Populations were analyzed and compared using random amplified polymorphic DNA and sixteen primers. Results of study showed that two populations of *G. rostochiensis* appeared to be distinct from the main group of this species as well as one population of *G. pallida*. Distinct clusters were observed within both species but the clusters could not be related to the geographic proximity of the populations.

AFLP

Amplified Fragment Lenght Polymorphism (AFLP) is a fingerprinting technique used to detection of polymorphism in different genomic regions of DNA. This method, highly sensitive and reproducible, is based on the PCR amplification of restriction fragments from a total digest of genomic DNA (Zabeau and Vos, 1993). It based on the ligation of adapters to ends of restriction fragments and a selective PCR-based amplification with adapter-specific primers. The standard procedures of AFLP described by Vos *et al.* (1995) become widely used for the identification of genetic variation in closely related species and populations. Like RFLP and RAPD analysis, AFLPs give an information about mutation that are dispersed over the genome. The comparative study of *Globodera* species and populations using AFLP revealed greater inter- and intra-

specific variability than obtained by RAPD (Subbotin and Moens, 2006). AFLP technique were used to amplify genomic DNAs extracted from cysts of 16 Swedish and 20 other European populations of PCN, both *G. rostochiensis* and *G. pallida* from UK, Germany, Norway and the Netherlands (Manduric and Andersson, 2003). The results of analysis revealed that Swedish Ro1 populations were very similar to corresponding populations from other parts of Europe. The rest of *G. rostochiensis* populations appeared as a genetically heterogeneous group with two Swedish populations being most dissimilar. Characteristic of 9 *G. rostochiensis* populations carried out by Folkertsma *et al.* (1996) by AFLP analysis clustering them into three groups based on variants expressed by the presence or absence of polymorphic DNA fragments. These groups were distinguished by 3,7 and 12 unique DNA fragments, respectively.

Microsatellites

Microsatellites such as Simple Sequence Repeats (SSR) or Short Tandem Repeats (STR) or Simple Sequence Length Polymorphisms (SSLP) are repetitive DNA in which short DNA motifs are repeated many times in tandem (TRs). They are unstable and can mutate at rates between 10^3 and 10^6 per cell (Gemayel *et al.*, 2012). TRs are present in coding and non-coding regions of nematode genome (Castagnone-Sereno *et al.*, 2010; Pérez-Jiménez *et al.*, 2013; Phumichai *et al.*, 2015). Particular alleles differ in number of tandem repeats and give an information about differences in nematode populations (Jarne and Lagoda, 1996). In 2013 Boucher *et al.* with the used of three sets of microsatellite markers was looking for genetic diversity and the origin of introduction of 15 populations of *G. rostochiensis* distributed worldwide. Obtained results confirm influence of genetic drift on losing of genetic diversity of tested nematode populations and indicate populations from South America less diverse than European ones.

Microsatellites markers were also used to explain of allelic richness of *G. pallida* populations introduced from South America to Europe, Africa, North America and Asia. Plantard *et al.* (2008) carried out comparative analysis of the allelic richness at seven microsatellite loci observed in the Western European populations. They found only one-third of them observed in this part of southern Peru comparable to the allelic richness observed in the northern region of Peru. The authors give an explanation that genetic variability can be a result of a single introduction of infected plant and can influence on control of quarantine nematodes on the field.

Many molecular techniques based on DNA/RNA analysis is used to searching for variations among nematode populations but for practical use there is a need to distinguish species and pathotypes. Shields *et al.* (1996) used the polymerase chain reaction to amplify a region between the 5S rRNA and spliced leader RNA genes in *G. rostochiensis* and *G. pallida*. Isolates of *G. rostochiensis* amplified a single 914 bp product and were distinguishable from *G. pallida* isolates which amplified 914 and 853 bp products or a single 853 bp product. Concordant identifications of *G. pallida* and *G. rostochiensis* isolates were obtained by using 5S-SL PCR for specific DNA probes and differential

host plant tests. Later the multiplex PCR analysis with primers GroR-GroF and PaR-PaF has been developed to distinguish the species *Globodera rostochiensis* and *G. pallida* by Fullaondo *et al.* (1999). Using a approach based on melting peak analysis of PCR products Bates *et al.* (2002) developed a semi-quantitative assay to measure the relative proportions of *Globodera pallida* and *G. rostochiensis* in a sample. The method depends on a multiplex PCR where the products of each species can be separated by their individual melting temperatures (Tm). 2% of *G. pallida* cysts in a mixture could be detected. Nakhla *et al.* (2010) developed the multiplex real-time PCR assays for the identification of the potato and tobacco cyst nematodes. They used a set of primers (PITSpf and PITS4) and a TaqMan probe (GFAMp) for the specific detection of *G. pallida*, and another set of primers (PGrtf and Prostor) for the detection of *G. rostochiensis* and the specific for *G. tabacum* primer set (PGrtf and PITSt3mr) with a TaqMan probe (GTETp).

However DNA/RNA markers could not be used to distinguish the PCN pathotypes. Hinch *et al.* (1998) developed a technique of high performance capillary electrophoresis (CE) that allows to obtain polypeptide profiles of each of the pathotypes of *G. rostochiensis* Rol-5, and *G. pallida* Pal, and mixture of Pa2 and Pa3.

CONCLUSION

Understanding of genetic diversity between populations and way of its dispersal in- and outside of origin geographic region is essential to prevent further spreading and create new methods of protection strategies. Both host range and genetic variation are important to have access to methods that enable inter- and intra-species identification of nematode populations. Classical methods of identification of PCN population based on morphological characterization and biotests identification are time-consuming and are more often replaced by molecular methods which are independent of environmental influence and stage of nematode. DNA-based diagnostic techniques are relatively quicker and the results seems to be more reliable in assessments of similarity of populations. For the practical purposes it is possible to distinguish the species of the evaluated nematodes on the basis of the DNA/RNA markers but up to now it is not possible to distinguish the pathotypes in a such way.

REFERENCES

Armstrong M.R., Blok V.C., Phillips M.S. 2000. A multipartite mitochondrial genome in the potato cyst nematode *Globodera pallida*. *Genetics* 154: 181-192.

Armstrong M.R., Husmeier D., Phillips M.S., Blok V.C. 2007. Segregation and recombination of a multipartite mitochondrial DNA in population of the potato cyst nematode *Globodera pallida*. *Journal of Molecular Evolution* 64: 689-701.

Avise J., Nelson C., William S., Charles G. 1994. Why one-kilobase sequences from mitochondrial DNA fail to solve the hoatzin phylogenetic enigma. *Molecular and Phylogenetics Evolution* 3(2): 175-184.

Baldwin J.G., Mundo-Ocampo M. 1991. Heteroderidae, cyst- and no-cyst forming nematodes. In: W. R. Nickle (Ed.), *Manual and Agricultural Nematology*, New York, Marcel Dekker, pp. 275-362.

Baldwin J. G. 1992. Evolution of cyst and non-cyst forming *Heteroderidea*. *Annual Review of Phytopathology* 30: 271-290.

Bate J. A., Taylor E. J., Gans P.T., Thomas J.E. 2000. Determination of relative proportions of *Globodera* species in mixed populations of potato cyst nematodes using PCR product melting peak analysis. *Molecular Plant Pathology* 3(3): 153-161.

Bendezu I. F., Evans K., Burrows P.R., de Pomerai D., Canto-Saenz M. 1998. Inter and intra-specific genomic variability of the potato cyst nematodes *Globodera pallida* and *G. rostochiensis* from Europe and South America using RAPD-PCR. *Nematologica* 44: 49-61.

Birky C.W. Jr. 2008. Uniparental inheritance of organelle genes. *Current Biology* 18: 692-695.

Blok V.C, Phillips M.S., Harrower B.E. 1997. Comparison of British populations of potato cyst nematodes with populations from continental Europe and South America using RAPDs. *Genome* 40: 286-293.

Blok V.C, Malloch G., Harrower M.S., Vrain T.C. 1998. Intraspecific variation in ribosomal DNA in population of the potato cyst nematode *Globodera pallida*. *Journal of Nematology* 30(2): 262-274.

Bossis M., Mugniery D. 1993. Specific status of six *Globodera* parasites of *solanaceous* plants studied by means of two-dimentional gel electrophoresis with comparison of gel patterns by a computer system. *Fundamental and Applied Nematology* 16: 47-56.

Boucher A.C., Mimee B., Bardou-Valette S., Belair G., Moffett P., Greiner E. 2013. Genetic diversity of the golden potato cyst nematode *Globodera rostochiensis* and determination of the origin of populations in Quebec, Canada. *Molecular and Phylogenetic Evolution* 69(1): 75-82.

Bridge J. 1992. Nematodes. In: R. J. Hillocks (Ed.), *Cotton diseases*, Walling Ford, UK: CSB International, pp. 331-353.

Burrows P.R., Boffey S.A. 1986. A technique for the extraction and restriction endonuclease digestion of total DNA from *Globodera rostochiensis* and *Globodera pallida* second-stage juveniles. *Revue de Nématologie* 9: 199–200.

Castagnone-Sereno, P., Danchin E.G.J., Deleury E., Guillemaud T., Malausa T., Abad P. 2010. Genome-wide survey analysis of microsatellite in nematodes, with the focus on the plant-parasitic species *Meladogyne incognita*. *BMC Genomics* 11: 598.

Chrisanfova G.G., Charchevnikov D.A., Popov I.O., Zinovieva S.V., Semyenova S. K. 2008. Genetic variability and differentiation of three Russian populations of potato cyst nematode *Globodera rostochiensis* as revealed by nuclear markers. *Russian Journal of Genetics* 44(5): 533-538.

Da Conceição I.L., Santos M.C.V., de Oliviera Abrantes I.M., de Almeida Santos M.S. 2003. Using RAPD markers to analyze genetic diversity in Portuguese potato cyst nematode populations. *Nematology* 5: 137 -143.

de Jong A.J., Bakker J., Roos M., Gommers F.J. 1989. Repetitive DNA and hybridization patterns demonstrate extensive variability between siblings species *Globodera rostochiensis* and *G. pallida*. *Parasitology* 99: 133-138.

Evans K., Franco J., de Scurrah M.M. 1975. Distribution of species of potato cyst nematodes in South America. *Nematologica* 21: 365-369.

Ferris V.R., Ferris J.M. 1992. Integration of classical and molecular approaches in nematode systematics. In: F. J. Gommers and P. W. Th. Maas (Eds.), Nematology from Molecule to Ecosystems, *European Society of Nematologists*, Inwergowrie, Dundee, Scotland, pp. 92-100.

Ferris V.R., Ferris J.M., Faghihi J. 1991. Biological diversity from the perspective of molecular biology. In: D. C. Dudley (Ed.), The Unity of Evolutionary Biology, Portland, OR, Dioscorides Press, pp. 71-76.

Ferris V.R., Ferris J.M., Faghihi J. 1993. Variation in spacer ribosomal DNA in some cyst forming species of plant parasitic nematodes. *Fundamental and Applied Nematology* 16: 177-184.

Folkertsma R.T., Rouppe van der Voort J.N., van Gent-Pelzer M.P.E., de Groot K.E., van den Bos W.J.R., Schots A., Bakker J., Gommers F.J. 1994. Inter- and Intraspecific variation between populations of *Globodera rostochinesis* and *G. pallida* revealed by Random Amplified Polymorphic DNA. *Genetics*, **84**(8): 807-811.

Folkertsma R.T., Rouppe van der Voort J.N., de Groot K.E., van Zandvoort P.M., Schots A., Gommers F.J., Helder J., Bakker J. 1996. Gene pool similarities of potato cyst nematode populations assessed by AFLP analysis. *Molecular Plant-Microbe Interaction* 9: 47-54.

Fullaondo A., Barrena E., Viribay M., Barrena I., Salazar A., Ritter E. 1999. Identification of potato cyst nematode species *Globodera rostochiensis* and *G. pallida* by PCR using specific primer combinations. *Nematology* 1: 157–163.

Gemayel R., Cho J., Boeynaems S., Verstrepen K.J. 2012. Beyond junk-variable tandem repeats as facilitators of rapid evolution of regulatory and coding sequences. *Genes* 2012(3): 461–480.

Greiner E., Bossis M., Fouville D., Renault L., Mugniery D. 2001. Molecular approaches to the taxonomic position of Peruvian potato cyst nematodes and gene pool similarities in indigenous and imported populations of *Globodera*. *Heredity* 86: 277-290.

Harrison R.G., 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology and Evolution* 4: 6-11.

Hinch J.M., Alberdi F., Smith S.C., Woodward J.R., Evans K. 1998. Discrimination of European and Australian *Globodera rostochiensis* and *G. pallida* pathotypes by high performance capillary electrophoresis. *Fundamental and Applied Nematology* 21(2): 123-128.

Hlaoua W., Horrigue-Raouani N., Fouville D., Mugniery D. 2008. Morphological and molecular characterization of potato cyst nematode populations from Tunisia and survey of their probable geographical origin. *Biotechnology* 7(4): 651-659.
Hoolahan A.H., Blok V.C., Gibson T., Dowton M. 2012. A comparison of three molecular markers for the identification of population of *Globodera pallida*. *Journal of Nematology* 44(1): 7-17.
Hyman B.C., Powers T.O. 1991. Integration of molecular data with systematics of plant parasiting nematodes. *Annual Review of Phytopathology* 28: 89-107.
Innan H., Nordborg M. 2002. Recombination or mutational hot spots in human mtDNA?. *Molecular Biology Evolution* 19: 1122-1127.
Jarne P., Lagoda P.J. 1996. Microsatellites, from molecules to population and back. *Trends in Ecology and Evolution* 11(10): 44-429.
Kivisild T., Villems R., Jorde L.B., Bamshad M., Kumar S., Hedrick P., Dowling T., Stoneking M., Parsons T.J., Irwin J.A., Awandalla P., Eyre-Walker A., Smith A. 2000. Questioning evidence for recombination in human mitochondrial DNA. *Science* 288: 1931a.
Kort J., Ross H., Rumpenhorst H.J., Stone A.R. 1977. An international scheme for identifying and classifying pathotypes of potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Nematologica* 23: 333-339.
Manduric S., Andersson S. 2003. Potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) from Swedish potato cultivar – an AFLP study of their genetic diversity and relationships to other European populations. *Nematology* 5(6): 851-858.
Nakhl, M.K., Owens K.J., Li W., Wei G., Skantar A.M., Levy L. 2010. Multiplex real-time PCR assays for the identification of the potato cyst and tobacco cyst nematodes. *Plant Disease* 94(8): 959-965.
Nicol J.M. Turner S.J., Coyne D.L., den Nijs L., Hockland S., Maafi Z.T. 2011. Current nematode threats to world agriculture. In: J. T. Jones, G. Gheysen and C. Fenoll (Eds.), *Genomics and Molecular Genetics of Plant-Nematode Interactions*, Heidelberg, Germany, pp. 21-44.
Nowaczyk K., Dobosz R., Budziszewska M., Kamasa J., Obrępalska-Stęplowska A. 2011. Analysis of diversity of golden potato cyst nematode (*Globodera rostochiensis*) populations from Poland using molecular approaches. *Journal of Phytopathology* 159: 759-766.
Oro V., Oro Radovanovic V. 2012. Molecular characterization of PCN populations from Serbia. *Genetika* 44 (1): 189-200.
Pastrik K.H., Rumpenhorst H.J., Burgermeister W. 1995. Random amplified polymorphic DNA analysis of a *Globodera pallida* population selected for virulence. *Fundamental and Applied Nematology* 18(2): 109 -114.
Perez-Jimenez M., Besnard G., Dorado G., Hernandez P. 2013. Varietal tracing of virgin olive oils based on plastid DNA variation profiling. *PLoS One* pp. 8.
Phillips M. S., Harrower B.E., Trudgill D.L., Waugh R., Catley M.A. 1992. Genetic variation in British populations of *Globodera pallida* as revealed by isozyme and DNA analysis. *Nematologica* 38: 301-319.
Phumichai C., Phumichai T., Wongkaew A. 2015. Novel chloroplast microsatellite (cpSSR) markers for genetic diversity assessment of cultivated and wild *Hevea* rubber. *Plant Molecular Biology Reporter* 33: 1486–1498.
Plantard O., Pickard D., Valette S., Scurahh M., Greiner E., Mugniery D. 2008. Origin and genetic diversity of Western European populations of the potato cyst nematode (*Globodera pallida*) inferred from mitochondrial sequences and microsatellite loci. *Molecular Ecology* 17(9): 2208-2218.
Pont-Kingdon G.A., Okada N.A., Macfarlane J.L., Beagley C.T., Wolstenholme D.R. 1995. A coral mitochondrial *mut*S gene. *Nature* 375: 109-111.
Pypylenko L.A., Phillips M.S., Blok V.C., 2008. Characterization of two Ukrainian populations of *Globodera pallida* in terms of their virulence and mtDNA, and the biological assessment of a new resistant cultivar Vales Everest. *Nematology* 10: 585-590.
Powers T.O., Adams B. 1993. Nucleotide sequences in nematode systematics. In: F. Lamberti, C. DeGiorgi and D. McK. Bird (Ends.), *Advances in Molecular Plant Nematology*, London, UK. Plenum, pp. 9-118.
Powers T. 2004. Nematode molecular diagnostics: from bands to barcodes. *Annual Review of Phytopathology* 42: 367-383.
Schnick D., Rumpenhorst H.J., Burgermeister W. 1990. Differentiation of closely related *Globodera pallida* (Stone) populations by means of DNA restriction fragment length polymorphisms (RFLPs). *Journal of Phytopathology* 130: 127-136.
Shield R., Fleming C.C., Stratford R. 1996. Identification of potato cyst nematode using the polymerase chain reaction. *Fundamental and Applied Nematology* 19(2): 167-174.
Subbotin S.A., Halford P.D., Warry A., Perry R. 2000. Variation in ribosomal DNA sequences and phylogeny of *Globodera* parasitizing solanaceous plants. *Nematology* 2(6): 561-604.
Subbotin S.A., Moens M. 2006. Molecular taxonomy and phylogeny. In: R. N. Perry and M. Moens (Eds.), Plant Nematology, Wallingford, UK. *CAB International*, pp. 40-721.
Thiery M., Mugniery D. 2000. Microsatellite loci in the phytoparasitic nematode *Globodera*. *Genome* 43(1): 160-165.
Vos P., Hogers R., Blekker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. 1995. AFLP: a new technique to DNA fingerprinting. *Nucleic Acids Research* 23(21): 4407-4414.

Welsh J., McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18: 7213-7218.
Zabeau M., Vos P. 1993. Selective restriction fragment amplification: A general method for DNA fingerprinting. *European Patent Application*, No.: 92402629.7. Publication number EP 0534858.

PLANT BREEDING AND SEED SCIENCE
Volume 80 2019

DOI: 10.37317/ pbss-2019-0009

Parvin Salehi Shanjani[1]*, Amir Mousavi[2], Faeze Rasoulzadeh Farsad[3]

[1] Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization, P.O. Box 13185-116, Tehran, Iran; [2] National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; [3] Islamic Azad Universities, Tehran, Iran; Correspondent author e-mail: psalehi1@gmail.com

SEED GERMINATION PLASTICITY OF TWO ENDANGERED SPECIES OF *FERULA* IN THE CONTEXT OF CLIMATE CHANGE

ABSTRACT

Ferula assa-foetida and *F. gummosa*, Apiaceae, are important endemic and endangered medicinal plants. Survival of the species is threatened by climate change, overexploiting (as source of oleo-gum resin and forage) and lack of organized cultivation. Cultivation of these valuable medicinal plants is restricted by insufficient domestication knowledge. Germination characteristics of different populations of *Ferula* taxa were studied with the aim of describing and comparing their responses to continuous cold stratification condition. Germination cues for the species were complex, with dormancy mechanisms present to restrict germination until cold stratification are fulfilled. Results indicated that a period of 4 weeks of stratification is sufficient for germination of *F. assa-foetida*, but optimal germination of *F. gummosa* require stratification for periods of 8 weeks. Both species were able to germinate at very low temperatures (4°C). Within-taxon differences in dormancy breaking and seedling emergence may interpret as local adaptations. The continued regeneration and propagation of the species in the wild will depend on the temperature and moisture status of the soil during winter and the maintenance of conditions suitable for stratification for an appropriate length of time.

Key words Dormancy, global warming, Iran, highland, local adaptation

INTRODUCTION

Seed germination is a critical stage in the life cycle of plants, particularly when considering the effects of global warming on high-altitude species. This is due to the dependence of these species on specific temperature regimes to stimulate germination and ensure seedling development coincides with favorable growing conditions (Mondoni *et al.* 2008, 2011; Milbau *et al.* 2009). The germination emergence stage is a high-risk phase of the plant life cycle, and there-

Communicated by Grzegorz Żurek

fore seed-based research can be useful in identifying species at risk of extinction from climate change, i.e. species with a narrow germination niche in terms of temperature range and/or stratification requirement (Cochrane *et al.* 2011; Walck *et al.* 2011). Information of this type provides a link between environmental change and the mechanisms that control population processes (Ooi *et al.* 2009; Cochrane *et al.* 2011; Walck *et al.* 2011; Ooi 2012), and can thus help to improve the accuracy of models predicting plant response to climate change (Ooi 2012).

Ferula assa-foetida and *F. gummosa*, Apiaceae, are important endemic and endangered medicinal plants. The taxa are monocarpic, herbaceous and perennials spread at altitudes of 1500–2500 m, with an average annual precipitation of 350–700 mm of Iran (Safaian and Shokri 1993; Mozaffarian 1996; Ivan 2007; Amiri and Joharchi 2016). Recently, survival of the species is threatened by climate change, overexploiting (as source of oleo-gum resin and forage) and lack of organized cultivation. Cultivation of these valuable medicinal plants is restricted by edaphic and climatic factors, low percentage of seed set and seasonal dormancy, and insufficient domestication knowledge (Golmohammadi *et al.* 2016).

According to Baskin and Baskin (2014), linear embryos in the Apiaceae are under-developed, and seeds have morphological dormancy (MD) or morpho-physiological dormancy (MPD). Normally, seeds with MD only need suitable temperature, moisture, oxygen, and of course time to germinate (Baskin and Baskin 2014). However, in many cases, the fully differentiated under-developed embryos also have physiological dormancy (PD), which imposes an additional constraint to germination; such embryos do not germinate in less than one month in suitable germination conditions. In this case, the dormancy is not just morphological but morphophysiological (MPD), and the embryos require additional treatment, such as cold, to complete their growth. Previous studies have classified *F. gummosa*, and *F. asafetida* as having deep morphophysiological dormancy, since cold stratification had been suggested as the main dormancy-breaking treatment (Otroshi *et al.* 2009; Rouhi *et al.* 2012). Formation of deep MPD seems to be an adaptation to regions with a very cold winter and a dry, cool summer. In these areas, temporary sporadic favorable temperatures (elevated temperature) in winter or too early in winter are threatening for seedling establishment. Therefore, the dormancy helps seeds to remain ungerminated throughout the winter. Moreover, the low temperatures alleviate dormancy and once dormancy breaks, two possible scenarios might occur: non-dormant seeds either wait for a mild and moist spring to germinate, or they germinate at low temperatures in the middle of winter in cold soil, even covered with heavy snow, until late winter; while the shoots grow and emerge above the soil surface with the increase in temperature (Baskin and Baskin 2014).

Different dormancy breaking and germination stimulating treatments have been tried with seeds of many species of Apiaceae (Baskin and Baskin 1991; Baskin *et al.* 1992, 1995, 1999, 2000; Nadjafi *et al.* 2006; Amooaghaie 2009; Nowruzian *et al.*,, 2016; Fasih and Tavakkol Afshari 2018). Results of different treatments including various levels of gibberellic acid, HNO3, chilling and soaking with water at different temperatures showed that moist-chilling and gib-

berellic acid treatments seem the most promising in *Ferula* species. The best treatments for *F. assa-foetida* was moist-chilling for 4 weeks at 5 ± 1 °C or for 2 weeks of moist-chilling (at 5 ± 1 °C) followed by soaking GA3 (10 mgL^{-1}) solution for 24 h (Nowruzian *et al.*,, 2016). In similar way treatment of moist-chilling for 6 weeks or 4 weeks followed by 500 ppm gibberellic acid is recommended for *F. ovina* (Fasih and Tavakkol Afshari 2018). Washing and chilling (5±1C) for a period of 14 days was most effective in breaking dormancy in *F. gummosa* (Nadjafi *et al.* 2006).

According field observations of authors, cold stratification causes embryos to complete growth and germinate in the middle of winter in cold soil or covered with heavy snow. Shoots grow and emerge above the soil surface following increasing of temperature in early spring. Therefore aims of the present work were stimulating the cold stratification (the treatment that occurs in nature), study of dormancy termination time and seedling growth in *F. assa-foetida*, *F. gummosa*.

Differences of the present work with earlier are in unlimited cold stratification duration for dormancy breaking; and exposing moist chilling condition for seedling growth. Moreover, studied differences and similarities among closely related taxa in order to increase understanding of adaptations and changes in seed dormancy and germination preferences. One difficulty when comparing seed dormancy and germination between taxa is the intra-taxon variation. Variation within a taxon may depend on genetic differences, local weather during growth of mother plants and maturation of seeds, seed position on the mother plant, soil quality, or other naturally occurring factors. To be able to draw conclusions on a general level, for example for modeling or predicting changes in emergence pattern following climate change, knowledge about a taxon, including its variation, is needed. Therefore for investigation of the impact of the habitat variability, germination characteristics among different populations of *F. assa-foetida* and *F. gummosa* were studied under continuous moist chilling conditions. Information about germination can also improve the success rate of using seed for rehabilitation, which is critical to restoration of the high altitude rangelands.

MATERIALS AND METHODS

Seed material of 23 accessions or populations of the two *Ferula* taxa from all over Iran were obtained from Natural Resources Gene Bank, Iran (Table 1).

For each accession 150 seeds were sterilized with 70% ethyl alcohol for five minutes, and then washed with distilled water. Three replicates (50 seeds per replicate) of sterilized seed were placed in Petri dishes on double Whatman papers (TP). For protection against moulds, the water used to moisten the seed samples and substrata contained 0.002% Binomial fungicide. The samples were immediately transferred into a germinator at 4±1°C and 12/12 h light (400 lux)/ dark for 60 days. Once the seeds started to germination, the number of germinated seeds were recorded every two days until the end of the experiment (two months). The length of roots and shoots of 10 randomly-selected seedlings

from each replicate were measured in 30 days seedlings. After measuring shoot and root lengths, the caryopses were cut from the seedlings and fresh seedling weight of each replicate was recorded. The seedlings were then placed in an oven at 80°C for 24 hours, after which the dry weight of each replicate was recorded as a percentage of the fresh weight. The vigor index measures seedling performance, relating together the germination percentage and growth of seedlings produced after a given time (Abdul-Baki and Anderson 1973).

Table 1

Some details of the studied wild *Ferula* populations

Province	Population	Code	Latitude (decimal)	Longitude (decimals)	Altitude (m above sea level)	Mean annual precipitation (mm)
			F. assa-foetida			
Horm	Bandar Abbas	FaBandarA1	28.17	56.83	2200	178
Horm	Bandar Abbas	FaBandarA2	27.88	50.22	1845	179
Khor	Boshroye	FaBoshroye	33.96	57.17	893	94
Horm	Haji Abad	FaHajiAbad	28.94	56.46	1900	179
Esfah	Kashan	FaKashan	33.75	51.48	1800	137
Kerm	Kerman	FaKerman	30.09	57.76	2300	133
Fars	Lar	FaLar	27.46	54.39	2000	200
Yazd	Mehriz	FaMehriz	33.36	57.34	1565	84
Yazd	Tabas	FaTabas1	33.39	57.26	1536	56
Yazd	Tabas	FaTabas2	31.52	54.32	2090	56
Yazd	Taft	FaTaft	31.66	54.18	2122	60
Kerm	Zarand	FaZarand	30.88	56.88	2300	47
			F. gummosa			
K&B	Dena	FgDena	30.50	51.72	2560	760
Elam	Elam1	FgEelam	33.63	46.41	1000	575
Horm	Haji Abad	FgHajiAbad	28.12	56.84	2200	178
Ch B	Lordegan	FgLordegan	31.42	51.26	2683	555
Semn	Shahrod	FgShahrod	35.87	56.65	950	139
Yazd	Tabas	FgTabas	33.36	57.34	1565	84
Yazd	Taft	FgTaft	31.56	54.16	2439	60
K&B	Yasuj	FgYasuj1	30.48	51.79	2300	855
K&B	Yasuj	FgYasuj2	31.94	51.44	1950	855
K&B	Yasuj	FgYasuj3	30.45	51.65	2420	855
Kerm	Zarand	FgZarand	30.88	56.87	2400	47

Horm - Hormozgan; Khor - Khorasan; Esfah - Esfahan; Kerm - Kerman; K&B - Kohkeluye and Boyerahman; Ch B - Charmahal Bakhtiali; Semn - Semnan

Data analysis

Variance analysis (ANOVA) were conducted for seed germination traits including dormancy termination, germination period, germination percentage, germination rate, germination index, seed vigor index, radicle length [mm], shoot length [mm], seedling length [mm], radicle/shoot length ratio, seedling fresh weight [mg], seedling dry weight [mg], seedling dry matter %; and seed morphology traits including seed weight [g], seed length [mm], seed width [mm], and 1000 seeds weight [mg] using the SAS9 software (SAS Institute Inc). To assess the relationships among the 13 different traits Pearson's correlation coefficient was analyzed using statistical analysis system software (SAS version 9.1, SAS Institute, 2001). The standardized morphological data were employed to calculate the Euclidean distances among the 23 *Ferula* populations by NTSYS-pc version 2.1 (Rohlf, 2002). Moreover, unweighted pair group methods of arithmetic mean (UPGMA) algorithm and SAHN clustering were utilized to get the genetic relationships. The Principal component analysis (PCA) of 23 *Ferula* populations was determined by Minitab software (version 15).

RESULTS

Seeds length, width and weight of *F. assa-foetida* (in length: 8 – 15 mm; in width: 4 – 7.7 mm; in weight: 9 – 23 mg) and *F. gummosa* (in length: 9-15 mm; in width: 6.5-10 mm; in weight: 7.7-32 mg) ranged among populations of each species (Table 2). ANOVA suggested significant differences among wild populations of *Ferula* species for the seed traits. A relatively high CV was obtained for seed weight (Table 2).

Table 2

Mean comparisons of seed morph characteristics of 23 populations of *Ferula assa-foetida* (with prefix Fa) and *F. gummosa* (with prefix Fg) constant cold stratification. Different letters indicate significant differences among different populations for the same species. P <0.05

Population	Seed weight [mg]		Seed length [mm]		Seed width [mm]	
			F. assa-foetida			
FaBandA1	12.23	c-f	9.73	f	5.47	c
FaBandA2	13.57	cd	9.30	f	5.67	c
FaBoshro	13.00	cde	15.17	a	7.65	a
FaHajiAb	8.90	f	7.68	g	4.12	d
FaKashan	16.93	b	11.77	bcd	6.97	b
FaKerman	23.00	a	12.21	bc	6.95	b
FaLar	15.23	bc	12.40	b	5.93	c
FaMehriz	9.23	f	11.23	cd	5.77	c
FaTabas1	10.33	def	10.07	ef	5.93	c
FaTabas2	10.13	ef	10.98	ed	5.87	c
FaTaft	10.33	def	11.82	bcd	6.80	b
FaZarand	17.77	b	12.08	bc	6.72	b
Mean	13.37		11.19		6.15	
Cv	43.86		16.95		16.6	

Table 2

Continued

Population	Seed weight [mg]		Seed length [mm]		Seed width [mm]	
			F. gummosa			
FgDena	20.07	bc	12.60	b	7.30	bc
FgEelam	15.00	d	12.53	b	6.40	ef
FgHajiAb	14.70	d	10.72	cd	5.97	f
FgLordeg	32.17	a	14.75	a	7.60	b
FgShahro	22.20	b	12.75	b	10.08	a
FgTabas	7.70	e	9.92	d	6.50	def
FgTaft	6.17	e	8.87	e	5.32	g
FgYasuj1	19.77	bc	12.87	b	7.10	bcd
FgYasuj2	22.10	b	13.33	b	7.18	bc
FgYasuj3	17.00	cd	12.43	b	6.40	ef
FgZarand	15.57	d	11.13	c	6.75	ced
Mean	17.49		11.99		6.96	
Cv	37.51		17.10		16.36	

Both species *F. assa-foetida* and *F. gummosa*, failed to germinate without prior stratification. However, cold stratification stimulated the germination and growth of seedlings of both species. ANOVA suggested significant differences among wild populations of *Ferula* species for all the seed germination traits. A relatively high CV was obtained for germination period, germination rate, seed vigor index, seedling fresh weight and seedling dry weight; moderate to low values of CV were obtained for the remaining traits (Table 3). Comparison of means verified that the duration of dormancy termination was significantly longer in *F. gummosa* (ranged from 31-51 days, with average 42 days) than *F. assa-foetida* (ranged from 12-28 days, with average 19 days) (Table 3; Fig. 1). Different populations of *F. assa-foetida* species had the significantly higher germination period, germination percentage, germination rate, germination index, seed vigor index and radicle length values (Table 3). In the species *F. assa-foetida* the highest germination characteristics (germination percentage, rate and index) were obtained in the population Fa-Tabas1and the highest seedling parameters (radicle and shoot length, and seedling fresh weight) were observed in the populations FaTaft and FaZarand; however, these two populations showed lowest values of seedling dry matter percentage. In the species *F. gummosa* the highest germination characteristics (germination percentage, rate and index) were obtained in the populations FgLordegan and FgTaft and the highest seedling parameters (radicle and shoot length, and seedling fresh weight and seedling dry matter percent) were obtained in the population FgYasuj2 (Table 3). Populations FgYa-suj1, 2, 3 of the species F. gummosa, with similar habitat and geographical range, have markedly different dormancy and germination characters (Table 3). Variation within a taxon may depend on genetic differences, local weather during growth of mother plants and maturation of seeds, seed position on the mother plant, soil quality, or other naturally occurring factors.

Table 3

Mean comparisons of seed germination characteristics of 23 populations of *Ferula assa-foetida* (with prefix Fa) and *F. gummosa* (with prefix Fg) under constant cold stratification. Different letters indicate significant differences among different populations for the same species. P <0.05

Population	Dormancy termination [days]		Germination period [days]		Germination [%]		Germination rate		Germination Index		Seed vigor index		Radicle length [mm]	
							F. assa-foetida							
FaBandA1	14.33	d	10	ab	50.67	cd	8.427	bc	478.2	cd	42.9	abc	28.13	a
FaBandA2	16.33	cd	16.67	ab	70.67	abc	6.300	cde	472.3	cd	32.4	a-d	16.33	abc
FaBoshro	23	ab	10.67	ab	26.67	de	2.797	ed	220.4	de	14.9	dc	18.52	abc
FaHajiAb	13.67	d	9.33	ab	46.67	cde	7.337	bcd	433.6	cd	17.3	bcd	11.17	c
FaKashan	21.67	bc	20.67	a	66.67	abc	7.950	bcd	562.7	bc	46.8	ba	13.20	bc
FaKerman	28.33	a	20.67	a	58.67	abc	4.273	cde	390.6	cd	33.6	a-d	23.43	abc
FaLar	28.33	a	20.67	a	20.00	e	1.343	e	122.9	e	6.5	d	11.92	c
FaMehriz	26.33	ab	16.00	ab	62.67	abc	5.860	cde	481.8	cd	26.4	a-d	14.20	bc
FaTabas1	13.00	d	10.00	ab	86.67	a	15.190	a	824.5	a	39.8	abc	16.90	abc
FaTabas2	12.33	d	6.67	b	80.00	ab	14.430	a	771.6	ab	42.9	abc	21.10	abc
FaTaft	12.33	d	8.00	b	62.67	abc	11.630	ab	607.1	abc	53.4	a	22.40	abc
FaZaran	17.00	cd	13.33	ab	53.33	bcd	7.817	bcd	486.7	cd	46.4	abc	25.73	ab
Mean	18.89		13.56		57.11		7.780		487.7		33.6		18.59	
Cv	16.93		45.19		26.36		36.000		29.1		48.3		37.60	

Table 3

Continued

Population	Dormancy termination [days]		Germination period [days]		Germination [%]		Germination rate		Germination Index		Seed vigor index		Radicle length [mm]	
F. gummosa														
FgDena	45.00	bcd	7.33	cb	20.00	c	0.763	e	91.8	d	13.28	ef	22.99	cb
FgEelam	45.00	bcd	8.67	cb	30.67	c	1.133	de	138.5	d	12.98	ef	8.817	d
FgHajiAb	48.33	bc	4.00	c	69.33	ab	2.347	cd	295.8	c	29.25	cd	13.87	cd
FgLordeg	38.33	de	11.33	cb	78.67	a	3.940	ab	439.1	ab	69.25	a	23.13	cb
FgShahro	51.67	ab	4.67	c	29.33	c	0.823	e	106.7	d	13.03	ef	14.20	cd
FgTabas	35.00	e	9.33	cb	65.33	ab	4.110	ab	417.2	abc	33.72	cb	15.80	bcd
FgTaft	32.33	e	14.00	b	72.00	ab	5.080	a	489.6	a	25.48	cde	11.60	d
FgYasuj1	33.00	e	22.67	a	70.67	ab	2.767	bc	322.4	bc	43.42	b	25.20	b
FgYasuj2	56.33	a	2.667	c	16.00	c	0.253	e	36.0	d	16.93	def	42.10	a
FgYasuj3	43.00	cd	10.00	cb	18.67	c	0.800	e	93.3	d	6.27	f	9.67	d
FgZarand	31.67	e	14.67	b	56.00	b	3.577	bc	359.6	abc	26.12	cde	13.80	cd
Mean	41.79		9.94		47.88		2.330		253.6		26.34		18.29	
Cv	8.72		45.04		24.69		32.93		28.1		28.83		30.16	

Table 3

Continued

Population	Shoot length [mm]		Seedling length [mm]		Radicle/shoot length ratio		Seedling fresh weight [mg]		Seedling dry weight [mg]		Seedling dry matter [%]	
					F. assa-foetida							
FaBandA1	46.45	abc	74.58	ba	0.613	ab	34.67	ab	2.333	b	6.72	d
FaBandA2	29.73	cd	46.07	bcd	0.573	ab	20.73	ab	2.167	b	5.70	d
FaBoshro	37.95	bcd	56.47	a-d	0.503	abc	19.50	ab	2.157	b	11.84	b
FaHajiAb	29.49	cd	40.66	dc	0.397	abc	22.69	ab	7.623	a	5.80	d
FaKashan	55.03	ab	68.23	abc	0.253	c	34.00	ab	3.167	b	9.49	c
FaKerman	34.07	bcd	57.50	a-d	0.700	a	34.33	ab	2.833	b	8.40	c
FaLar	19.36	d	31.28	d	0.627	ab	17.98	b	2.987	b	16.57	a
FaMehriz	28.23	cd	42.43	dc	0.510	abc	25.26	ab	1.333	b	6.20	d
FaTabas1	27.57	cd	44.47	bcd	0.600	ab	13.80	b	1.233	b	9.83	c
FaTabas2	31.00	cd	52.10	bcd	0.653	ab	12.73	b	1.000	b	8.15	c
FaTaft	60.97	a	83.37	a	0.357	bc	29.13	ab	1.833	b	6.49	d
FaZaran	61.03	a	86.77	a	0.460	abc	40.63	a	2.400	b	6.07	d
Mean	38.41		56.99		0.520		25.46		2.590		15.35	
Cv	30.20		28.53		30.360		44.94		60.570		14.36	

Table 3

Continued

Population	Shoot length [mm]		Seedling length [mm]		Radicle/shoot length ratio		Seedling fresh weight [mg]		Seedling dry weight [mg]		Seedling dry matter [%]	
					F. gummosa							
FgDena	42.69	b	65.68	b	0.537	ab	36.40	b	4.067	c	11.53	bcd
FgEelam	36.44	cb	45.26	cd	0.260	b	17.31	cd	1.577	d	9.33	cde
FgHajiAb	28.40	c	42.27	d	0.540	ab	26.87	cb	1.167	d	4.35	f
FgLordeg	66.33	a	89.47	a	0.347	b	68.23	a	7.567	a	11.11	cd
FgShahro	28.59	c	42.78	d	0.483	ab	11.06	d	1.263	d	11.97	cb
FgTabas	36.03	cb	51.83	bcd	0.440	ab	17.90	cd	1.633	d	7.26	e
FgTaft	24.13	c	35.73	d	0.483	ab	17.20	cd	1.300	d	7.51	e
FgYasuj1	36.27	cb	61.47	cb	0.697	a	36.87	b	3.267	c	8.98	de
FgYasuj2	62.19	a	104.3	a	0.673	a	18.71	cd	5.980	b	31.03	a
FgYasuj3	35.81	cb	45.47	cd	0.273	b	21.86	cd	3.167	c	14.20	b
FgZarand	32.43	cb	46.23	cd	0.457	ab	19.33	cd	1.433	d	7.893	e
Mean	39.03		57.32		0.470		26.52		2.950		11.38	
Cv	17.17		16.72		32.750		28.44		27.740		13.34	

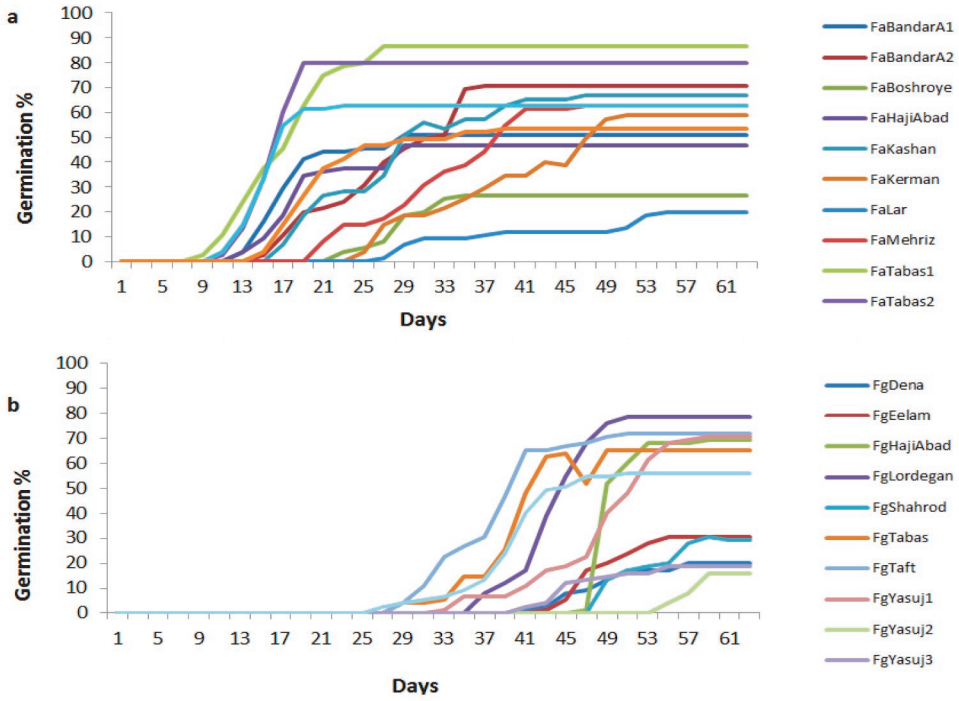

Fig. 1. Comparison of germination percentage of different populations of *Ferula assa-foetida* (a; with prefix Fa) and *F. gummosa* (b; with prefix Fg) under constant cold stratification

Using Pearson's correlation, an analysis was done to assess the relationship among the germination and seedling traits. It is useful to determine the relationship among the traits since this information will be useful in the utilization of the germplasm as well in the collection of the germplasm based on the target traits. The correlations among measured traits are shown in Table 4. Dormancy termination, as the most important trait, was positively and significantly correlated with important germination characters including germination percentage, germination rate and seedling vigor index; and negatively correlated with all seed morphological characters. Germination percentage exhibited a positive and significant correlation with germination rate, germination index and seedling vigor index (Table 4; Fig. 2). The correlation analysis indicated that some phenotypic traits had significant correlation ($p \leq 0.05$) with climate factors (Table 5). The mean annual precipitation had positive correlation with dormancy termination time, seed weight and length; but showed negative correlation with the germination percentage, germination rate, and germination index. Seedling fresh weight had positive correlation with altitude. There was also positive correlation between seed width and latitude. The above correlations implied that the mean annual precipitation plays an important role in influencing the dormancy and germination traits of the *Ferula* taxa.

Table 4

Pair wise correlation between seed (germination and morph) characteristics of different populations of *Ferula* taxa

Characters	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.
1. Dormancy termination	1.000															
2. Germination period	-0.346	1.000														
3. Germination%	-0.497*	0.246	1.000													
4. Germination rate	-0.83**	0.013	0.678**	1.000												
5. Germination Index	-0.79**	0.166	0.863**	.944**	1.000											
6. Seed vigor index	-0.45*	0.178	0.778**	0.593**	.709**	1.000										
7. Radicle length	0.066	-0.188	-0.066	0.044	-0.034	0.335	1.000									
8. Shoot length	0.026	-0.172	-0.025	0.064	0.029	0.554**	0.634**	1.000								
9. Seedling length	0.044	-0.195	-0.044	0.063	0.007	0.521*	0.840**	0.952**	1.000							
10. Radicle/shoot length ratio	-0.077	0.130	0.100	0.083	0.059	-0.016	0.529**	-0.281	0.012	1.000						
11. Seedling fresh weight	-0.013	0.244	0.224	-0.053	0.067	0.638**	0.340	0.649**	0.589**	-0.149	1.000					
12. Seedling dry weight	0.139	-0.036	-0.216	-0.228	-0.242	0.085	0.327	0.450*	0.445*	-0.128	.537**	1.000				
13. Seedling dry matter%	-0.101	-0.040	-0.194	-0.075	-0.143	-0.274	0.032	-0.092	-0.052	0.052	-0.169	0.533**	1.000			
14. Seed weight	0.506*	0.053	-0.222	-0.464*	-0.430*	0.170	0.397	0.481*	0.494*	0.019	0.623**	0.503*	-0.025	1.000		
15. Seed length	0.432*	-0.005	-0.405	-0.446*	-0.478*	-0.005	0.318	0.437*	0.432*	-0.050	0.353	0.173	-0.318	0.700**	1.000	
16. Seed width	0.511*	-0.148	-0.293	-0.397	-0.410	-0.010	0.223	0.298	0.297	-0.043	0.126	-0.059	-0.308	0.640**	0.731**	1.000

*: significant at 0.05 level; **: significant at 0.01 level

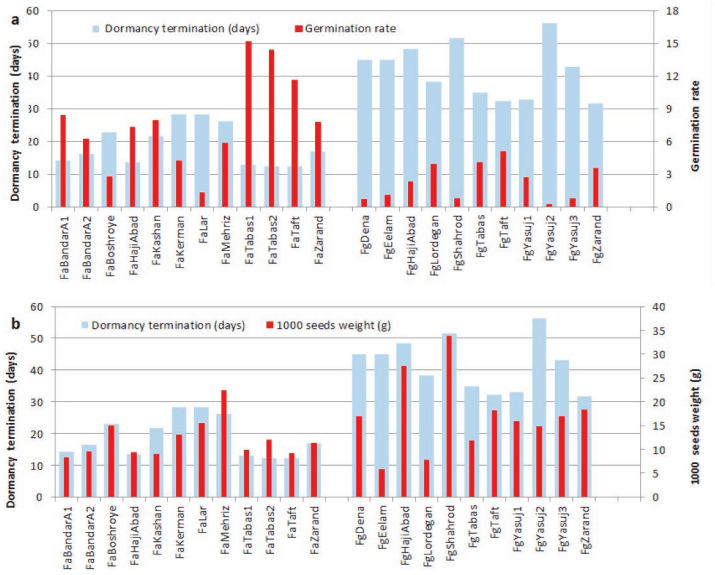

Fig. 2. Comparison of dormancy termination with germination rate (a) and with 1000 seeds weight (b) of different populations of *Ferula assa-foetida* (with prefix Fa) and *F. gummosa* (with prefix Fg)

Table 5

Pearson correlation analyses for the relationship between seed (germination and morph) characteristics within different populations of *Ferula* taxa and some ecological parameters

Parameters	Dormancy termination	Germination period	Germination [[%	Germination rate	Germination Index	Seed vigor index	Radicle length	Shoot length
Latitude	0.200	-0.218	-0.017	0.032	0.037	-0.002	-0.084	0.140
Longitude	-0.321	-0.028	0.203	0.286	0.307	0.052	-0.004	-0.265
Altitude	-0.028	0.195	0.224	0.037	0.116	0.361	0.275	0.264
Mean annual precipitation	0.61**	-0.112	-0.45*	-0.6**	-0.6**	-0.224	0.319	0.274
	Seedling length	Radicle/ shoot length ratio	Seedling fresh weight	Seedling dry weight	Seedling dry matter [[%	Seed weight	Seed length	Seed width
Latitude	0.065	-0.328	-0.218	-0.238	-0.345	0.016	0.343	0.574**
Longitude	-0.187	0.331	-0.157	-0.271	-0.247	-0.315	-0.263	-0.057
Altitude	0.294	0.131	0.56**	0.330	-0.079	0.262	-0.106	-0.263
Mean annual precipitation	0.319	-0.020	0.267	0.495*	0.155	0.535**	0.444*	0.206

*: significant at 0.05 level; **: significant at 0.01 level

The Euclidean distances matrix was subjected to agglomerative hierarchical clustering utilizing UPGMA method to construct a dendrogram (Fig. 3). 23 populations of the *Ferula* taxa were classified into two main groups. Cluster I consisted of 10 populations of *F. gummosa* and 4 populations of *F. assa-foetida*; cluster II included eight populations of *F. assa-foetida* and only one population of *F. gummosa* (Fig. 3). Comparison of means of two clusters indicated that populations in cluster I have significantly higher dormancy termination time, germination period and seed weight, however

populations cluster II showed higher germination percentage, germination rate, germination index, seed vigor index and seedling length (Table 6). UPGMA trees of seed germination andmorphological characters partially separated the two species, a behavior also supported by PCA plot (Fig. 4). However, almost within each species cluster, the populations differed somewhat from each other and were joined together with different distances.

Therefore, there was no obvious relationship between phenotypic traits and the origin of these *Ferula* populations. PCA analysis of seed germination and morphological data revealed that the first 4 components comprise about 77% of total variance (Table 7). The first component accounted for 34.4% of the total variation in the data set while the second and third principal components contributed 21.2% and 14.4%, respectively. Together, these three components could explain 68% of the total variation in the characterized the *Ferula* populations. Analysis of the factor loadings of the characters in the retained PCs indicated that any of seed germination and morphological traits showed positive loadings in PC 1-3 (Table 7).

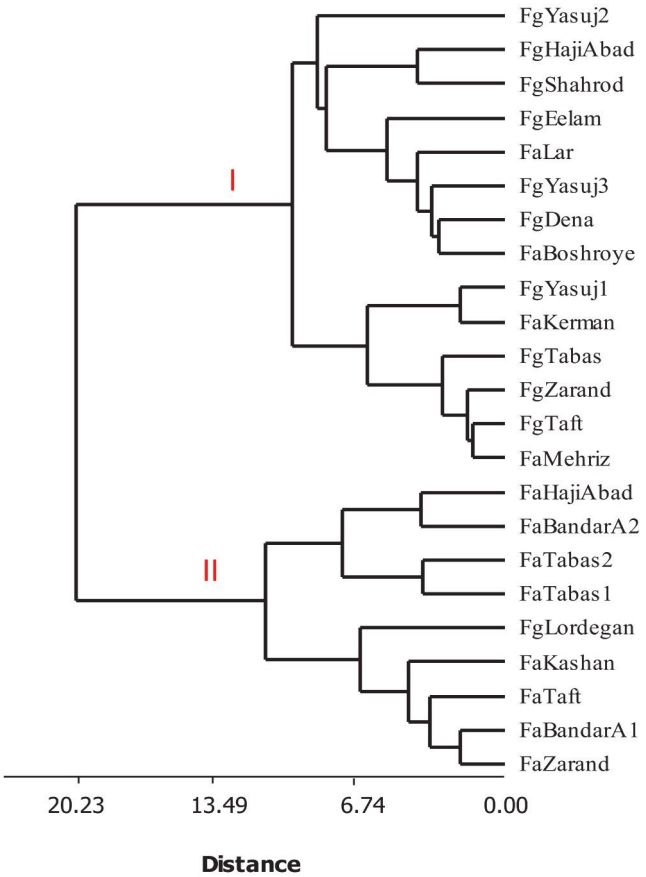

Fig. 3. Dendrograms of the 23 populations of *Ferula assa-foetida* (with prefix Fa) and *F. gummosa* (with prefix Fg) based on studied traits

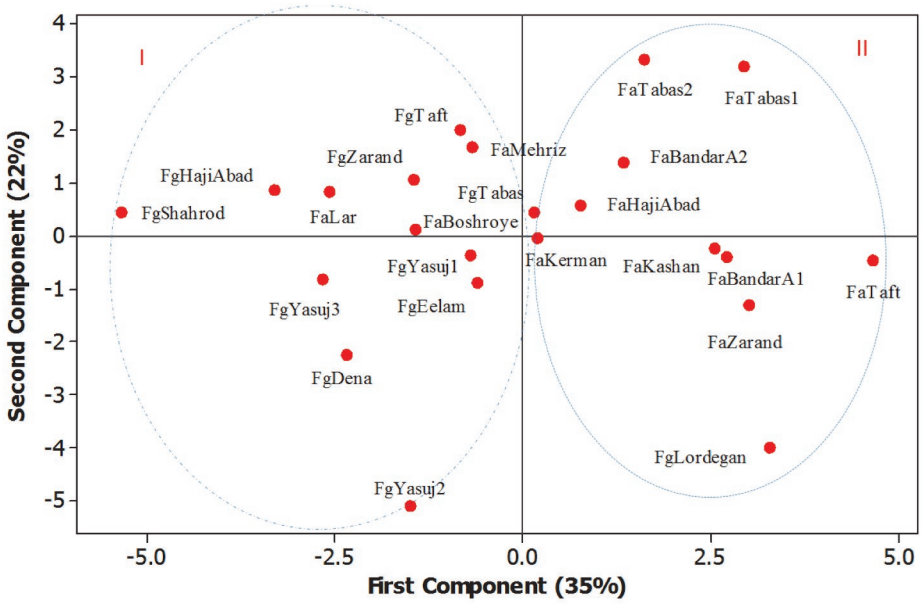

Fig. 4. Scatter diagram of the 23 populations of *Ferula assa-foetida* (with prefix Fa) and *F. gummosa* (with prefix Fg) based on studied traits

Table 6

Mean comparisons of seed (germination and morph) characteristics of populations that separated in two clusters of Fig. 4. Different letters indicate significant differences among different populations for the same species (P ≤0.05)

Group	Dormancy termination [days]	Germination period [days]	Germination [%]	Germination rate	Germination Index	Seed vigor index	Radicle length [mm]	Shoot length [mm]
I	36.85a	12.40a	46.53b	2.78b	274.18b	22.80b	17.55a	34.14b
II	17.28b	11.67a	66.70a	9.38a	570.14a	42.43a	19.45a	43.71a
	Seedling length [mm]	Radicle/ shoot length ratio	Seedling fresh weight [mg]	Seedling dry weigh [mg]	Seedling dry matter [%]	Seed weight [mg]	Seed length [mm]	Seed width [mm]
I	51.69b	0.52a	23.18b	2.38a	10.78b	18.26a	12.17a	6.87a
II	63.15a	0.48a	29.41a	3.23a	18.04a	10.72b	10.77a	6.03a

Table 7

Factor loadings (eigenvectors) for the different seed characteristics of the *Ferula* populations for the principal components retained

Variable	PC1	PC2	PC3	PC4
Dormancy termination	-0.295	-0.267	0.048	0.174
Germination period	0.068	0.105	-0.028	0.204
Germination [[%	0.236	0.24	0.271	0.177
Germination rate	0.308	0.268	0.101	-0.133
Germination Index	0.31	0.285	0.154	0.01
Seed vigor index	0.323	-0.03	0.312	0.197
Radicle length	0.105	-0.3	0.363	-0.345
Shoot length	0.214	-0.392	0.1	0.075
Seedling length	0.192	-0.393	0.214	-0.084
Radicle/shoot length ratio	-0.056	0.089	0.403	-0.438
Seedling fresh weight	0.172	-0.293	0.189	0.265
Seedling dry weight	0.057	-0.343	-0.1	-0.233
Seedling dry matter [%]	-0.005	-0.057	-0.296	-0.537
Seed weight	-0.322	0.067	0.274	-0.015
Seed length	-0.267	0.144	0.261	-0.131
Seed width	-0.263	0.069	0.315	-0.053
Eigenvalue	6.1832	3.8224	2.2375	1.6753
Proportion	0.344	0.212	0.124	0.093
Cumulative	0.344	0.556	0.68	0.773

DISCUSSION

Germination cues for *F. assa-foetida* and *F. gummosa* were complex, with dormancy mechanisms present to restrict germination until cold stratification or other requirements are fulfilled (Nadjafi *et al.* 2006; Amooaghaie, 2009; Nowruzian *et al.* 2016; Fasih and Tavakkol Afshari, 2018). The existence of morphophysiological dormancy (MPD) is very frequent in the Apiaceae (Baskin *et al.* 1992, 1995, 2000; Phartyal *et al.* 2009; Vandelook *et al.* 2008, 2009; Scholten *et al.* 2009; Yaqoob and Nawchoo 2015; Fasih and Tavakkol Afshari 2018). Cold stratification temperature used in this experiment (4°C) provides an adequate moist chilling treatment. The temperature is also within the range of

soil temperatures likely to be encountered in the field in high altitude Iran (Tabari and Talaee 2011; Ghasemi, 2015; Aghajanlou and Ghorbani 2016; Shirvani *et al.* 2018). This cold stratification temperature has been reported as successful in breaking dormancy in studies of alpine and high altitude species (Baskin and Baskin 2014).

Results indicated that the duration of dormancy termination was significantly longer in *F. gummosa* than *F. assa-foetida*. A period of 4 weeks of stratification is sufficient for germination of *F. assa-foetida*, but *F. gummosa* require cold stratification for periods of 8 weeks for optimal germination. The final germination percentage of *Ferula* taxa at present study was higher than the previous experiences (Nadjafi *et al.* 2006; Amooaghaie 2009, Nowruzian *et al.* 2016; Fasih and Tavakkol Afshari 2018), in which *Ferula* seeds transferred to standard germination condition following limited cold stratification treatment. Sommerville *et al.* (2013) by studding of several species of Australian Alps suggested species requiring stratification for periods of 8 weeks or more for optimal germination may be particularly sensitive to climate change. High altitude ecosystems are considered to be among the most sensitive to climate changes (Hughes 2003; Laurance *et al.* 2011), and recent declines in average snow depth have been observed in alpine and high altitude areas in both the Northern and Southern Hemispheres (Hughes 2003; Nicholls 2005; Hennessy *et al.* 2007; Rosenzweig *et al.* 2007; Amiri and Eslamian 2010). For species in Apiaceae depend on cold moist conditions (wet stratification) to break dormancy; reduced snow cover during winter may threaten the survival of these species, even if subsequent temperatures are suitable for germination (Liu *et al.* 2011). Although the seed of some species may be able to tolerate winter temperatures in the absence of snow, a reduction in snow cover may also mean a reduction in the amount of available water (in total precipitation in winter and spring). As the level of seed hydration plays a role in breaking seed dormancy (Hoyle *et al.* 2008; Walck *et al.* 2011; Baskin and Baskin 2014), relative drought during winter and spring may prove to be more important in limiting the germination of these species than the lack of snow cover *per se* (Liu *et al.* 2011). Results of this research also indicated significant correlation between precipitation and germination traits.

Both species were able to germinate at very low temperatures (4°C). The ability to germinate at very low temperatures has been observed in several high altitude species (Wardlaw *et al.* 1989; Sommerville *et al.* 2013). The capacity to germinate at low temperatures may provide an advantage during a short growing season by allowing germination to begin under snow banks (Meyer *et al.* 1995; Forbis and Diggle 2001; Walck and Hidayati 2004). *Aciphylla glacialis* (Apiaceae) germinated optimally at low temperatures, similar to the Asian and North American *Osmorhiza* species (Walck *et al.* 2002; Baskin *et al.* 1995; Walck and Hidayati 2004) in the same family (Apiaceae). Cold stratification response having similar effects to high altitude and alpine species: improving final germination, widening the range of temperatures for germination, decreasing germination time, and synchronizing germination by reducing variability in time to germination (Shimono and Kudo 2005).

The study species were highly variable in their dormancy and germination response to the moist chilling treatment. Variation of the dormancy termination duration parameter was significant among different populations of each species; ranging from 31 to 51 days in the *F. gummosa*, and from 12 to 28 days in the *F. assa-foetida*. Dormancy is a genetic seed characteristic, but it strongly interacts with environmental factors. Dormancy intensity depends on age, nutritive conditions and water supply of the plant, as well as the weather conditions during seed ripening (Andersson and Milberg 1998). Ecological factors, such as temperature, humidity, oxygen and light, greatly influence the seed's dormancy period interruption, and there is a significant distinction of causes of dormancy discontinuance among species (Podrug *et al.* 2014; Mahmoudi *et al.* 2015; Mazangi *et al.* 2016; Mirzaei Mossivand *et al.* 2018; Aghajanlou *et al.* 2018). In concordance with the researches significant correlation were found between germination characteristics (including dormancy termination) and precipitation.

The germination responses of *F. assa-foetida*, *F. gummosa* seeds was significantly affected by seed origin. Several studies have been published of attempts to interrelate the germination responses of populations of a particular species collected in different parts of its range. Haasis and Thrupp (1931) and Skordilis and Thanos (1995) working with coniferous species, and McNaughton (1966) with *Typha* species all reported variations in germination of different ecotypes. Lauer (1953), on the other hand, failed to distinguish notable differences between populations of *Agrostemma githago* and *Datura stramoniam* collected in various locations in Europe. The variety of observed responses to germination is expected, as high altitude environments exhibit significant spatio-temporal variability (Kaye 1997; Shimono and Kudo 2005; Noroozi *et al.* 2013, 2015). Even within a particular habitat, germination responses are unlikely to be consistent. For each species, germination is likely to vary between altitudes and populations. Variability in germination is an important strategy to ensure species survival in unpredictable environments, reducing the risk of exposing the entire seedling cohort to poor growing conditions (Giménez-Benavides *et al.* 2005; Venn, 2007; Mondoni *et al.* 2008). For example, in the genus *Penstemon*, Meyer (1995) suggests that germination of most species combines predictive mechanisms (e.g. fulfillment of cold stratification requirements) with the potential for development of a persistent seed bank.

CONCLUSIONS

Cold stratification is the main prerequisite for breaking deep complex dormancy in *F. assa-foetida* and *F. gummosa*. A period of 4 weeks of stratification is sufficient for germination of *F. assa-foetida*, but *F. gummosa* require stratification for periods of 8 weeks for optimal germination. Both species were able to germinate at very low temperatures (4°C). The characteristics of deep MPD in the taxa are part of the plant's adaptation to its environment. Highly significant intraspecific population differences in the germination parameters of the taxa might reflect local adaptation to a particular environment. Pronounced differences occurred within both *F. assa-foetida* and *F. gummosa*, even though

the some studied sites in each taxon were adjacent sites. Variation within a taxon may depend on genetic differences, local weather during growth of mother plants and maturation of seeds, seed position on the mother plant, soil quality, or other naturally occurring factors. To be able to draw conclusions on a general level, for example for modelling or predicting changes in emergence pattern following climate change, knowledge about a taxon, including its variation, is needed. Therefore, studies of germination behavior should include several populations from the same species.

The continued regeneration of the species in the wild will depend on the temperature and moisture status of the soil during winter and the maintenance of conditions suitable for stratification for an appropriate length of time. In this context temperature is a critical driver of plant regeneration, directly influencing seed dormancy, germination and vegetative reproduction. Therefore changing climate not only affect the dormancy and germination traits, but also is likely to impact on the germination response of these species through maternal effects on the developing seed. These species could be targeted for conservation in *ex situ* collections, whilst monitoring their response in the field.

ACKNOWLEDGEMENTS

This work was supported by the Agricultural Research, Education and Extension Organization, and Research Institute of Forests and Rangelands (RIFR), Iran; Project no. 14-09-09-9354-93198.

REFERENCES

Abdul Baki A. A., Anderson J. D. 1973. Vigor determinations in soybean seed multiple criteria. Crop Sci. 13: 630-633.

Aghajanlou F., Ghorbani A. 2016. A study on the effects of some environmental factors on the distribution of *Ferula gummosa* and *Ferula ovina* in Shilander mountainous rangelands of Zanjan. *Iran J. Rangeland.* 9: 407-419.

Aghajanlou F., Ghorbani A., Zare Chahoki M. A., Hashemi Majd K., Mostafazadeh R. 2018. The impact of environmental factors on distribution of *Ferula ovina* (Boiss.) Boiss. in northwest Iran. *Appl. Ecol. Environ. Res*. 16: 977-992.

Amiri M. S., Joharchi M. R. 2016. Ethnobotanical knowledge of Apiaceae family in Iran: A review. Avicenna J. Phytomed. 6: 621-635.

Amiri M. J., Eslamian S. 2010. Investigation of Climate Change in Iran. J. Environ. Sci. Technol. 3: 208-216. doi:10.3923/jest.2010.208.216

Amooaghaie R. 2009. The Effect Mechanism of Moist-Chilling and GA on Seed Germination and Subsequent Seedling Growth of *Ferula ovina* Boiss. Open Plant Sci. J. 3: 22-28.

Andersson L., Milberg P. 1998. Variation in seed dormancy among mother plants, populations and years of seed collection. Seed Sci. Res. 8: 29–38.

Baskin C. C., Baskin J. M. 1991. Non-deep complex morphophysiological dormancy in seeds of *Osmorhiza claytonii* (Apiaceae). Amer. J. Bot. 78: 588-593.

Baskin C., Baskin J. M. 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, San Diego: Elsevier/Academic Press.

Baskin C. C., Baskin J. M., Chester E. W. 1999. Seed dormancy in the wetland winter annual *Ptilianium nuttalli* (Apiaceae). Wetland. 19: 23-29.

Baskin C. C., Chester E. W., Baskin J. M. 1992. Deep complex morphophysiological dormancy in seeds of *Thaspium pinnatifidum* (Apiaceae). Int. J. Plant Sci. 153: 565–571.

Baskin C. C., Meyer S. E., Baskin J. M. 1995. Two types of morphophysiological dormancy in seeds of two genera (*Osmorhiza* and *Erythronium*) with an arctotertiary distribution pattern. Amer. J. Bot. 82: 293–298.

Baskin C. C., Milberg P., Andersson L., Baskin J. M. 2000. Deep complex morphophysiological dormancy in seeds of *Anthriscus sylvestris* (Apiaceae). *Flora*. 195: 245–251.

Cochrane A., Daws M. I., Hay F. R. 2011. Seed-based approach for identifying flora at risk from climate warming. Austral. Ecol. 36: 923–935.

Fasih M., Tavakkol Afshari R. 2018. The morphophysiological dormancy of *Ferula ovina* seeds is alleviated by low temperature and hydrogen peroxide. Seed Sci. Res. 28: 52 –62. doi:10.1017/ S0960258517000356.

Forbis T. A., Diggle P. K. 2001. Subnivean embryo development in the alpine herb *Caltha leptosepala* (Ranunculaceae). Can. J. Bot. 79: 635–642.

Ghasemi A. R. 2015. Changes and trends in maximum, minimum and mean temperature series in Iran. Atmos. Sci. Lett. 16: 366–372.

Giménez-Benavides L., Escudero A., Pérez-Garcia F. 2005. Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability. Ecol. Res. 20: 433–444.

Golmohammadi F., Ghoreyshi S. E., Parvaneh H. 2016. *Ferula assa-foetida* as a main medical plant in east of Iran (harvesting, main characteristics and economical importance). Int. J. Farm. Alli. Sci. 5-6: 453-475.

Haasis F. W., Thrupp A. C. 1931. Temperature relations of lodgepole pine seed germination. *Ecology*. 12: 728 -744.

Hennessy K., Fitzharris B., Bates B. C., Harvey N., Howden S. M., Hughes L., Salinger J., Warrick, R. 2007. Australia and New Zealand. In: M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, C. E. Hanson (Eds.) *Climate change: impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change*, (pp. 507–540). Cambridge: Cambridge University Press.

Hoyle G. L., Daws M. I., Steadman K. J., Adkins S. W. 2008. Mimicking a semi-arid tropical environment achieves dormancy alleviation for seeds of Australian native Goodeniaceae and Asteraceae. Ann. Bot. 101: 701–708.

Hughes L. 2003. Climate change and Australia: trends, projections and impacts. Aust. Ecol. 28: 423–443.

Ivan A. R. 2007. *Ferula assafoetida*. Med. Plant World. 3: 223-234.

Kaye T. N. 1997. Seed dormancy in high elevation plants: implications for ecology and restoration. In: T. N. Kaye, A. Liston, R. M. Love, D. L. Luoma, R. J. Meinke, M. V. W ilson (Eds.) *Conservation and management of native plants and fungi*, (pp. 115–120). Eugene, OR: Native Plant Society of Oregon.

Lauer E. 1953. Über die Keimtemperatur von Ackerun-kräutern und deren Einfluß auf die Zusammensetzung von Unkrautgesellschaften. *Flora*. 140: 551-595.

Laurance W. F., Dell B., Turton S. M., Lawes M. J., Hutley L. B., McCallum H., Dale P., Bird M., Hardy G., Prideaux G., Gawne B., McMahon C. R., Yu R., Hero J. M., Schwarzkopf L., Krockenberger A., Douglas M., Silvester E., Mahony M., Vella K., Saikia U., Wahren C. H., Xu Z., Smith B., Cocklin C. 2011. The 10 Australian ecosystems most vulnerable to tipping points. Biol. Conserv. 144: 1472–1480.

Liu K., Baskin J. M., Baskin C. C., Bu H., Liu M., Liu W., Du G. 2011. Effect of storage conditions on germination of seeds of 489 species from high elevation grasslands of the eastern Tibet Plateau and some implications for climate change. Amer. J. Bot. 98: 12–19.

Mahmoudi J., Mahdavi S. Kh., Mansouri B. 2015. Examination of effect of topography (elevation and aspect) on distribution of medicinal plant *Ferula gummosa* case study: rangelands of Khombi and Saraii Germeh city in Khorasan Shomali Province. Bull. Environ. Pharmacol. Life Sci. 4: 108-113.

Mazangi A., Ejtehadi H., Mirshamsi O., Ghassemzadeh F., Hosseinian Yousefkhani S. S. 2016. Effects of climate change on the distribution of endemic *Ferula xylorhachis* Rech.f. (Apiaceae: Scandiceae) in Iran: Predictions from ecological niche models. Russ. J. Ecol. 47: 349-354, doi:10.1134/ s1067413616040123.

McNaughton S. J. 1966. Ecotype functions in the *Typha* community-type. Ecol. Monographs. 36: 297–325.

Meyer S. E., Kitchen S. G., Carlson S. L. 1995. Seed germination timing patterns in intermountain *Penstemon* (Scrophulariaceae). Amer. J. Bot. 82: 377–389.

Milbau A., Graae B. J., Shevtsova A., Nijs I. 2009. Effects of a warmer climate on seed germination in the subarctic. Ann. Bot. 104: 287–296.

Mirzaei Mossivand A., Ghorbani A., Zare Chahoki M. A., Keivan Behjou F., Sefidi K. 2018. Compare the environment factors affecting the distribution of species *Prangos ferulacea* and *Prangos pabularia* in rangelands of Ardabil Province. *Iran. J. Range Desert Res.* 25: 200-210.

Mondoni A., Probert R., Rossi G., Hay F., Bonomi C. 2008. Habitat-correlated seed germination behavior in populations of wood anemone (*Anemone nemerosa* L.) from northern Italy. Seed Sci. Res. 18: 213–222.

Mondoni A., Probert R. J., Rossi G., Vegini E., Hay F. R. 2011. Seeds of alpine plants are short lived: implications for long-term conservation. Ann. Bot. 107: 171–179.

Mozaffarian V. 1996. A dictionary of Iranian plant names: Latin, English, Persian: Farhang Mo'aser, 505p.

Nadjafi F., Bannayan M., Tabrizi L., Rastgoo M. 2006. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. J. Arid Environ. 64: 542–547.

Nicholls N. 2005. Climate variability, climate change and the Australian snow season. Austral. Meteorol. Magazne. 54: 177–185.

Noroozi J., Akhani H., Breckle S. W. 2013. Biodiversity and phytogeography of the alpine flora of Iran. Biodivers. Conserv. doi:10.1007/s10531-007-9246-7.

Noroozi J., Dietmar M., Franz E. 2015. Diversity, distribution, ecology and description rates of alpine endemic plant species from Iranian mountains. Alp. Bot. 126. doi:10.1007/s00035-015-0160-4.
Nowruzian A., Masoumian M., Ebrahimi M., Bakhshi Khaniki G. 2016. Effect of Breaking Dormancy Treatments on Germination of *Ferula assafoetida* L. Iran. J. Seed Res. 3: 155-169, doi:10.29252/yujs.3.2.155.
Ooi M. K. J., Auld T. D., Denham A. J. 2009. Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. Glob. Change Biol. 15: 2375–2386.
Ooi M. K. J. 2012. Seed bank persistence and climate change. Seed Sci. Res. 22: S53–S60.
Otroshi M., Zamani A., Khodambashi M., Ebrahimi M., Struik P. C. 2009. Effect of exogenous hormones and chilling on dormancy breaking of seeds of asafoetida (*Ferula assafoetida* L.). J. Seed Sci. 21: 9–15.
Phartyal S. S., Kondo T., Baskin J. M., Baskin C. C. 2009. Temperature requirements differ for the two stages of seed dormancy break in *Aegopodium podagraria* (Apiaceae), a species with deep complex morphophysiological dormancy. Amer. J. Bot. 96: 1086–1095.
Podrug A., Gadžo D., Muminović Š., Grahić J., Srebrović E., Đikić M. 2014. Dormancy and germination of johnsongrass seed (*Sorghum halepense* L.). *Herbologia*. 14(2):1-10, doi: 10.5644/Herb.14.2.01.
Rohlf F. 2002. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System (2.1 Ed.), Department of Ecology and Evolution, State University of NY, Stony Brook.
Rosenzweig C., Casassa G., Karoly D. J., Imeson A., Liu L., Menzel A., Rawlins S., Root T. L., Seguin B., Tryjanowski P. 2007. Assessment of observed changes and responses in natural and managed systems. In: M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, C. E. Hanson, (Eds.), *Climate change 2007: impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change*. (pp. 79–131). Cambridge: Cambridge University Press,.
Rouhi H. R., Rahmati H., Saman M., Shahbodaghloo A. R., Karimi F. A., Moosavi S. A., Rezaei M. E., Karimi F. 2012. The effects of different treatments on dormancy-breaking of Galbanum seeds (*Ferula gummosa* Boiss). Int. J. Agric. Sci. 27: 598–604.
Safaian N. and Shokri M. 1993. Botanical and ecological study of species of the genus *Ferula* (Medicinal Plants) in Mazandaran province. Acta Hort. 333: 159-167.
SAS Institute. 2001. SAS InstituteSAS/Stat user's guide, Version 9.1 SAS Institute, Cary, NC, USA.
Scholten M., Donahue J., Shaw N. L., Serpe M. D. 2009. Environmental regulation of dormancy loss in seeds of *Lomatium dissectum* (Apiaceae). Ann. Bot. 103: 1091–1101.
Shimono Y., Kudo G. 2005. Comparisons of germination traits of alpine plants between fellfield and snowbed habitats. Ecol. Res. 20: 189–197.
Shirvani A., Moradi-Choghamarani F., Zand-Parsa S., Moosavi A. A. 2018. Analysis of long-term trends in air and soil temperature in a semi-arid region in Iran. Environ Earth Sci. 77: 173-177.
Skordilis A., Thanos C. 1995. Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*. Seed Sci. Res. 5: 151–160.
Sommerville K. D., Martyn A. J., Offord C. A. 2013. Can seed characteristics or species distribution be used to predict the stratification requirements of herbs in the Australian Alps? Bot. J. Linn. Soc. 172: 187–204.
Tabari H., Talaee P. H. 2011. Analysis of trends in temperature data in arid and semi-arid regions of Iran. Glob. Planet Change. 79: 1–10.
Vandelook F., Bolle N., Van Assche J. A. 2008. Seasonal dormancy cycles in the biennial *Torilis japonica* (Apiaceae), a species with morphophysiological dormancy. Seed Sci. Res. 18: 161–171.
Vandelook F., Bolle N., Van Assche J. A. 2009. Morphological and physiological dormancy in seeds of *Aegopodium podagraria* (Apiaceae) broken successively during cold stratification. Seed Sci. Res. 19: 115–123.
Venn S. E. 2007. Plant recruitment across alpine summits in south-eastern Australia. DPhil Thesis, LaTrobe University.
Walck J. L., Hidayati S. N., Dixon K. W., Thompson K., Poschlod P. 2011. Climate change and plant regeneration from seed. Glob. Change Biol. 17: 2145–2161.
Walck J. L., Hidayati S. N., Okagami N. 2002. Seed germination ecophysiology of the Asian species *Osmorhiza aristata* (Apiaceae): comparison with its North American congeners and implications for evolution of types of dormancy. Amer. J. Bot. 89: 829–835.
Walck J. L., Hidayati S. N. 2004. Germination ecophysiology of the western North American species *Osmorhiza depauperata* (Apiaceae): implications of preadaptation and phylogenetic niche conservatism in seed dormancy evolution. Seed Sci. Res. 14: 387–394.
Wardlaw I. F., Moncur M. W., Totterdell C. J. 1989. The growth and development of *Caltha introloba* F. Muell. II. The regulation of germination, growth and photosynthesis by temperature. Austral. J. Bot. 37: 291–303.
Yaqoob U., Nawchoo I. A. 2015. Conservation and cultivation of *Ferula jaeschkeana* Vatke: a species with deep complex morphophysiological dormancy. Proceedings of the National Academy of Sciences, India Section B: Biological, 1–11.

Content