Predicted Drop in Gene Diversity Over Generations in the Population Where the Fertility Varies Among Individuals

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Abstract

Gene diversity and inbreeding of seed crop over generations were derived and predicted as a function of fertility variation and population sizes in forest tree populations. Gene diversity was calculated in terms of group coancestry. Fertility differences were described by sibling coefficient, which is the probability that two genes originate from the same parent, compared to a situation where all parents give rise to the same number of offspring. Fertility data were collected in a finite population, and calculations were made for the case of a constant breeding population size. The change of status number could be associated with the sibling coefficient. Predictions over five generations showed that group coancestry and inbreeding accumulated fast at the first early shifts. Relative status number declined very fast over generations. The increase of inbreeding and group coancestry was accelerated by fertility variation, and the accumulation was slightly faster and higher if fertility of both genders varied than if maternal fertility was kept constant. Gene diversity decreased faster if fertility variation was large, and maintained higher if the effective population size was reasonably large. Breeding programs that use closely related genotypes lead to the decrease of effective number (i.e., drop in gene diversity) over generations and do not provide a sustainable long-term breeding strategy.

Key words: fertility variation, status number, group coancestry, inbreeding, gene diversity, seed orchard.

Introduction

Gene diversity of seed crop is mainly influenced by the level of kinship (LINDGREN and MULLIN, 1998) and by a difference of gamete production among parental genotypes (XIE *et al.*, 1994; KJÆR, 1996; BURCZYK and CHALUPKA, 1997). Variation in fertility is one of the major factors in evolution and genetic management of populations. Here, fertility is defined broadly as the ability of an individual to produce successful gametes; i.e., living offspring. Fertility variation among individuals causes the accumulation of relatedness and reduces the status effective number in the seed crop (KANG and LINDGREN, 1998; BILA *et al.*, 1999). So, attention should be paid to the fertility variation among parents to maintain sufficient gene diversity.

There are further factors that can affect mating among genotypes and thus the genetic composition of progeny, such as distance, wind direction, genetic incompatibilities, gene migration and so on. But, their assessment and modelling are difficult and challengeable.

The use of effective population number (status number) for monitoring the gene diversity of seed crop from orchard populations was discussed by LINDGREN and MULLIN (1998). Status number expresses the genetic situation of seed crops when the structure or fertility of the parental population is known. So, it is a characteristic of seed crops based on the property of parental population, but the status number itself does not tell about the condition of parental population. It also describes what proportion of parents is effectively involved in the production of progeny, and how much is the accumulated genetic drift raised by relatedness and fertility variation (KANG and LINDGREN, 1999).

The increase in inbreeding within the populations during successive generations of recurrent selection is potentially a major problem in long-term breeding programs (GEA *et al.*, 1997). Breeding and conservation programs that use populations (e.g., seed orchards and seed stands) with a low status effective number may lead to a loss of gene diversity in the plantations (LINDGREN *et al.*, 1996). One way to reduce the loss of gene diversity is to restrict the parental contribution, more likely maternal contribution than paternal, to the next generation (WEI, 1995). By keeping an almost equal contribution of genotypes, genetic relatedness is minimised and gene diversity is maintained high in the population (LINDGREN *et al.*, 1996).

The objectives of present study are to derive status number, gene diversity and inbreeding of seed crop, and to predict their development over generations considering fertility variation and population sizes. The effect of fertility variation on the gene diversity is also discussed.

Theory

Fertility variation

Fertility variation can be described by sibling coefficient (Ψ) that relates to coefficient of variation (CV) in fertility (see KANG and LINDGREN, 1998; it was designated as A). Here, the maternal and paternal fertilities are defined as the relative numbers of maternal and paternal gametes produced by an individual, respectively (cf. GREGORIUS, 1989). We assume that there is no correlation between maternal and paternal fertilities. (There was no evident correlation between estimated maternal and paternal fertilities in this study). The sibling coefficient (Ψ) can thus be estimated as (KANG and LINDGREN, 1999)

$$\Psi = N \sum_{i=1}^{N} p_i^2$$

$$= 0.25 \left(CV_m^2 + CV_p^2 \right) + 1$$

where N is the number of genotypes contributing to the gamete gene pool, p_i is the contribution of genotype i, and CV_m and CV_p are the coefficients of variation for maternal and paternal contribution, respectively. When equal amount of seed is collected from each genotype, the maternal fertility is constant ($CV_m = 0$). Fertility variation will then be a function of the variation of

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paternal fertility (CV_p) and the sibling coefficient (Ψ) can be described as

$$\Psi = 0.25\left(CV_p^2\right) + 1$$

The sibling coefficient equals 1 when all individuals are equally fertile. It increases with unbalanced parental contribution to the progeny. When the sibling coefficient equals 2, it means that relatedness and inbreeding of seed crops will build up over generations by double as much speed and amount as the reference population (see below).

Group coancestry and status number

Group coancestry (Θ) is the probability that two genes taken from a gene pool of population are identical by descent (COCKERHAM, 1967). When we look at the group coancestry over generations (t), it can be described as follows (BILA *et al.*, 1999)

$$\Theta_t = \frac{0.5}{N_t} + \left(1 - \frac{0.5}{N_t}\right)\Theta_{gametes}$$

The successful gametes of generation t-1 are the gene pool of generation t. The group coancestry of the gamete pool can be described as function of the parent inbreeding (F), fertility variation (Ψ) and census number (N) as

$$\Theta_{gametes} = \frac{0.5(1+F_{t-1})\Psi_{t-1}}{N_{t-1}} + \left(1 - \frac{\Psi_{t-1}}{N_{t-1}}\right)\frac{N_{t-1}\Theta_{t-1} - 0.5(1+F_{t-1})}{N_{t-1}-1}$$

Note that the gamete group coancestry is the same as the parents if they are unrelated, non-inbred and equally fertile.

Status number (N_s) is defined as half the inverse of group coancestry (Θ) (LINDGREN *et al.*, 1996).

$$N_{s(t)} = \frac{0.5}{\Theta_t}$$

Status number expresses how many ideal genotypes would give rise to the considered offspring, and it can also describe the accumulated genetic drift from the reference population to which the concepts inbreeding and coancestry refer (LINDGREN and MULLIN, 1998). Group coancestry (status number) and sibling coefficient can be associated to the classical concept, the variance effective population size ($Ne^{(v)}$), as follows

$$N_{e(t)}^{(v)} = \frac{\Psi_t}{2\Theta_t(\Psi_t - 1)}$$

Variance effective population size describes the chance of change in gene frequencies at a generation shift: that is, the change of status number over generations.

Inbreeding and gene diversity

Group coancestry of present generation becomes the inbreeding of the following under random mating (FALCONER and MACKAY, 1996). Thus, the group coancestry of gametes in the preceding generation becomes the expected inbreeding in the offspring as follows

$$F_t = \Theta_{gametes}$$

Gene diversity (GD) in each generation can be estimated from the group coancestry, relatively to a reference population as (LINDGREN and KANG, 1997)

$$GD_t = 1 - \Theta_t = 1 - \frac{0.5}{N_{s(t)}}$$

Including gene diversity, all concepts which concern identity by decent refer back to the reference population where all genes are unique by definition and individuals are unrelated and non-inbred.

Materials and Methods

Clonal archive considered as a hypothetical population

The clonal archive of Korean pine (*Pinus koraiensis* S. et Z.) is located at latitude 36° 30'N, longitude 126° 20'E in Suwon, Republic of Korea, and consisted of 180 genotypes. Trees originated from phenotypically selected plus trees over all the distribution area of Korea. They were propagated by grafting, and planted with the equal number of six ramets. It was established in 1983. Details of the population and data collection are given by KANG and LINDGREN (1999). We assumed that these initial 180 genotypes were unrelated and non-inbred (thus the reference population is the forest where the 180 genotypes were drawn from) and pollen contamination was negligible.

Considered population is that a number of genotypes (N=180) are selected from the reference population as parents, and they are placed in a seed orchard. Seeds are harvested from the seed orchard. Among the harvested seeds, a number of genotypes (N=180) are chosen at random to form a new seed orchard. The fertility of the 180 genotypes can be different from generation to generation. There is random mating of gametes (random fusion) in the seed orchard. To illustrate possible variations between generations, observations of fertility differences in each year were assumed to vary with three different scenarios: 1) maternal and paternal fertilities were constant over generations, 2) maternal fertility was kept constant by collecting the same number of seeds per genotype, while paternal fertility varied over generations as observed among years and 3) maternal and paternal fertilities varied over generations.

Fertility variation, status number and inbreeding

For each tree in the population, the numbers of female and male strobili were counted for five successive years from 1991 through 1995. In this study, these five years were assumed to represent five consecutive generations. Status number, group coancestry, inbreeding and gene diversity were calculated based on the fertility variation estimated for each of the five years as described earlier in the theory. Calculations were made for the case of a constant breeding population size of generations, where the breeding population was derived at random from the zygotes of the seed crop. For relationship between census (N) and status number (N_s), relative status number (N_r) was calculated as N_s/N.

Results and Discussion

Fertility variation

There was a large difference in gamete contributions to the seed crop among genotypes. The difference in male strobilus production was much larger than in female (*Table 1*). There was a general lack of flower production over the studied period. Some results related with the genetic parameters (e.g., genetic variance and heritability) are reported in HAN *et al.* (1997). The difference in male strobilus production among genotypes was very extreme. The differential gamete production may have important impact on gene diversity of seeds, as uneven production will cause a reduction of the N_s.

Fertility variation was estimated based on flowering assessment for the five successive years, assuming that pollination

Table 1. – Average number of strobili per graft, coefficient of variation (*CV*) and sibling coefficient (Ψ) for female and male strobilus production.

	1991		1992		1993		1994		1995	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Average	2.2	49.7	3.5	25.6	8.1	44.1	5.8	75.2	3.4	43.9
CV	1.455	3.665	1.233	3.331	1.167	2.777	0.888	2.494	1.125	3.766
Ψ*	3.12	14.43	2.52	12.09	2.36	8.71	1.79	7.22	2.27	15.18

*) Fertility variation estimated independently for the each gender; $\Psi = (CV)^2 + 1$.

success is a saturating positive function of strobilus production (ALLISON, 1990; SCHOEN and STEWART, 1987). The sibling coefficient (Ψ) varied between genders and among years. In 1994, the sibling coefficient for both genders was the smallest among the studied years, while the production of female strobili was not at a peak. Maternal fertility was most poor in 1991, and paternal fertility variation was very large in 1995.

The sibling coefficient (Ψ) has no dimension and expresses how much fertility varies among parents as the increase in the probability that sibs occur compared to the situation where fertility is equal across the population (KANG and LINDGREN, 1999). If $\Psi = 2$, for instance, there will be twice as many sibs as compared to the equal fertilities. The sibling coefficient (Ψ) carries the same information as the coefficient of variation (*CV*), but Ψ is based on a probabilistic aspect while *CV* is based on a variance aspect. So, Ψ could give better genetic meaning than *CV*.

The genetic base of seeds produced in good flowering years is much broader compared to that produced in a poor year (MATZIRIS, 1993; EL-KASSABY *et al.*, 1989; EL-KASSABY and REYNOLDS, 1990). In the present study, only one reproductive phase has been observed. There are many other phases between flowering and viable offspring, e.g., pollen or ovule development, sexual selection, cone development, embryo development, polyembryo competition, seed maturation, germination, and early seedling survival or competition (SIEGISMUND *et al.*, 1996). Even the timing of anthesis and the receptivity of female flowers may have an effect on the parental contribution to the seed crop (RUOTSALAINEN and NIKKANEN, 1989). However, the quantitative number of flowers is the most important contributing factor to genetic composition of seed crops, especially for the species where most trees have a short flowering period of less than a week, such as *Pinus densiflora* (JANG, 1993) and *Pinus sylvestris* (KÄRKKÄINEN and SAVOLAINEN, 1993).

In tree breeding programs, the first generation is generally established with (broad-) wide gene diversity. It also includes a large diversity on fertility. The genotypes with high fertility ability or better fertility in the population may contribute more for next generations. Even if no artificial selection is made, the genotype will drift for fertility. Thus, the later generations will probably have low variances on fertility, and thus the loss of gene diversity will be smaller over generations.

Status number and group coancestry

Status number (N_s) varied over five generations. Status number and relative status number (N_r) declined by generation (*Table 2* and *Figure 1*). The decrease was remarkably fast in the first generation shift. For the idealised population having equal fertility and constant population size, N_r were calculated as 0.50, 0.33, 0.25, 0.20 and 0.17 for the five successive generations, respectively (*Figure 1*).

For the practical situation, however, N_r was much smaller than for the idealised situation, mainly due to the large

Table 2. – Prediction of group coancestry (Θ), status number (N_s), relative status number (N_r), variance effective population size ($N_e^{(v)}$), inbreeding (F), and gene diversity (GD) in the future generations at constant population size (N=180) following random fusion of gametes.

	Maternal and paternal fertilities vary						Maternal fertility constant					
Generation	0 *	1	2	3	4	5	0 *	1	2	3	4	5
Ψ**	1.00	4.89	4.15	3.27	2.75	4.86	1.00	4.36	3.77	2.93	2.55	4.55
Θ	0.0028	0.0163	0.0277	0.0365	0.0439	0.0568	0.0028	0.0149	0.0252	0.0331	0.0400	0.0521
N_s	180	31	18	14	11	9	180	34	20	15	13	10
N_r	1.00	0.17	0.10	0.08	0.06	0.05	1.00	0.19	0.11	0.08	0.07	0.05
$N_e^{(v)}$	Infinite	39.8	24.4	19.3	18.0	11.3	Infinite	43.7	27.0	22.9	20.6	12.3
F	0.000	0.014	0.025	0.034	0.041	0.054	0.000	0.012	0.022	0.030	0.037	0.049
GD	0.997	0.984	0.972	0.964	0.956	0.943	0.997	0.985	0.975	0.967	0.960	0.948

*) Generation 0 can be seen as the initial population (180 genotypes) that is drawn from the reference population.
**) Sibling coefficient (Ψ) estimated from individual years was considered successively as the fertility variation over five generations.

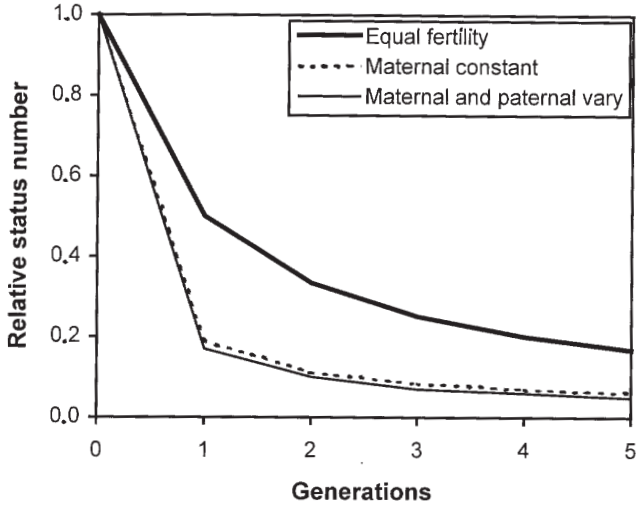

Fig. 1. – Decrease in relative status number (N_r) over five generations at constant population size (N= 180), different fertility variations (as observed in a breeding population) and following random fusion of gametes. Generation 0 can be seen as an initial breeding population consisting of unrelated and non-inbred genotypes.

fluctuations in male fertility. N_s was slightly higher when maternal fertility was kept constant than when both fertilities were varied (*Figure 1*). It was clear that the status number decreased as the fertility variation increased.

One can argue that fertility variation from poor flowering years couldn't represent the fertility variation over generations in this study. But, it has reported that there was a limited amount of flowering for many years in this species (HAN *et al.*, 1997; KANG and LINDGREN, 1999). So, the lack of flowering seems to be genetically controlled or a physiological property of this species. For the other species, however, it could be better to use fertility variation estimated from good or moderate flowering years, which could represent fertility variation in an evolutionary scale.

For quantification of gene diversity of seed crops, several concepts of effective population sizes have been used. The concept of effective population size is generally characterised by two traditional ways: inbreeding and variance effective population size, and by the status effective number. The traditional concepts are developed to describe a process, but the status number expresses a state of population based on relatedness (LINDGREN and MULLIN, 1998). KANG and LINDGREN (1999) also proposed the effective number of parents based only on the fertility variation among parents in the population. Status number describes the population as if it were so many unrelated and non-inbred genotypes, and also expresses the accumulated genetic drift from the reference population to which the concepts inbreeding and coancestry refer. Thus, N_r shown in the practical situation that 17%, 10%, 8%, 6% and 5% of the initial numbers of genotypes could be expected to contribute effectively to the seed crops (as unrelated and non-inbred genotypes) for the five successive years, respectively (*Figure 1* and *Table 2*). The decrease is due to the fertility variation.

The variance effective population sizes ($N_e^{(v)}$) as described here becomes infinite in the initial generation where there is equal fertility among genotypes (*Table 2*), reflecting that in a large seed crop from where parents are equally represented, the gene frequencies will be the same in the parents as in the progeny. When the sibling coefficient equals two ($\Psi = 2$), $N_e^{(v)}$ is double as high as status number over generations. But this relationship is not linear, depending on the Ψ values. Also, $N_e^{(v)}$ decreases as fertility variation increases, and the decrease has the same trend as status number when the generation shifted.

Group coancestry (Θ) and inbreeding (F) were increased over generations (*Figure 2* and *Table 2*). If there are N equally fertile founders in the generation 0 with N offspring, the group coancestry of founders (Θ_0) equals $0.5/N$, and that of next generation (Θ_1) equals $0.5/N+(1-0.5/N)(0.5/N)$. Therefore, the change of group coancestry (Θ_1/Θ_0) in the first generation shift is almost double as much as in the founder generation (*Figure 2*). Fertility variation and relatedness of founders will accelerate this increment, and it is shown that more than 80% of the decrease in N_s has occurred during the first generation turn over (*Figure 1*).

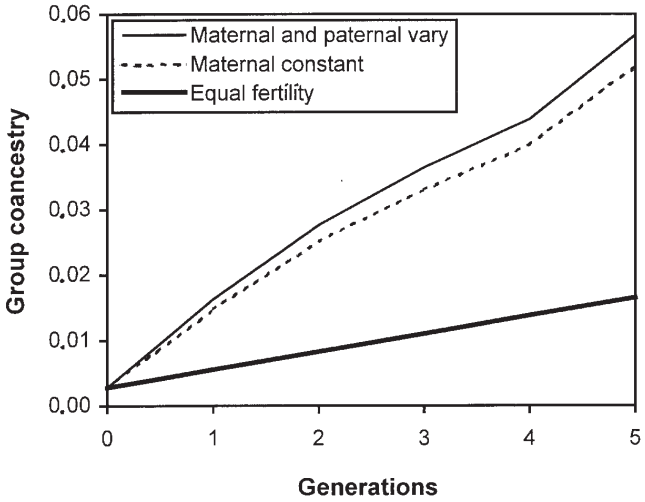

Fig. 2. – Accumulation of group coancestry (Θ) over five generations, assuming that the population size is constant (N= 180) and the new generation is formed by random mating.

The accumulation of inbreeding and group coancestry was faster and higher when the fertility variation was larger (*Table 2* and *Figure 2*). By keeping maternal fertility constant, inbreeding and group coancestry of seeds could be improved. However, the improvement was small because the fertility variation among maternal parents was small while paternal fertility variation was extremely large. The improvement would be remarkable if the paternal fertility could be kept equally (data not shown). This option would also save more seeds than controlling female fertility.

There was no correlation between genders in this study. But high correlation may act strongly to prevent the loss of gene diversity by the equal seed harvest, depending also on how fertility varies. For instance, BILA *et al.* (1999) reported that gene diversity decreased at a considerably slower rate when the contributions of one gender were kept constant, but the maternal and paternal fertility variations were similar and highly correlated in that investigation.

The coancestry of two individuals is the probability that two gametes taken at random, one from each, carry alleles that are identical by descent (FALCONER and MACKAY, 1996). Thus, the coancestry of any two individuals is identical with the inbreeding coefficient of their progeny if they were mated. Group coancestry is the expected average coefficients of inbreeding among the offspring following random mating, and it increases with number of individuals per genotype. This tendency will also be pronounced with increasing numbers of generations (MÜLLER-STARCK, 1982) and with variation in fertility. On the other hand, pollen contamination will increase N_s and *GD* (HARJU, 1995; LINDGREN and MULLIN, 1998).

Inbreeding and gene diversity

There was very low inbreeding when compared to the reference population over generations, indicating that the gene diversity of the seed crop was maintaining high over generations if individuals mate randomly (*Table 2* and *Figure 3*). So, if we establish plantations from the seed crops produced over generations, the increase of inbreeding in the seeds from the forest would not seem alarming.

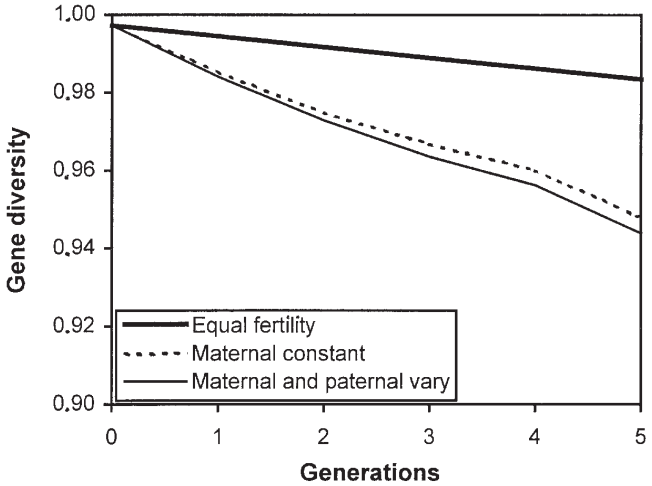

Fig. 3. – Gene diversity ($GD_t = 1\text{-}\Theta_t = 1\text{-}0.5/N_{s(t)}$) over five generations at the constant population size (N= 180) and with fertility variation for each generation.

The accumulation of inbreeding (and also group coancestry) was a bit faster and higher when the fertilities of both genders were varying than when the maternal fertility was constant (*Figure 3*). KJÆR (1996) reported that the accumulation of inbreeding was high when the flowering was poor. So, the loss of gene diversity or genetic variability will be mitigated when parental fertility is close to balance or when maternal fertility is kept constant.

Increase of inbreeding and reduction of gene diversity depend also on the effective population sizes. For all level of fertility variation, the gene diversity (*GD*) was decreasing dramatically when the effective population size was getting smaller than 10 (KANG *et al.*, 2001). GEA *et al.* (1997) reported that population size was an important factor for delaying inbreeding, and N_s was slightly better preserved by small disconnected groups over generations than large populations. But, the population should be large enough because a small status number in a population will reduce the gene diversity and adaptive potential of the progeny.

Direct assays of effective parental fertility have only been possible using biochemical markers such as isozymes (WHEELER and JECH, 1992). However, isozyme markers show less genetic variation in most operational populations, and thus are limited in their ability to apply an estimate of fertility variation (JOLY and ADAMS, 1985; EL-KASSABY *et al.*, 1989). Using isozyme marker, within population gene diversity estimates in conifers range from 0.0 to 0.35 (HAMRICK *et al.*, 1992), while polymorphic cpDNA markers yield a gene diversity estimation of 0.91 (STOEHR *et al.*, 1998).

In the ideal random mating population, the inbreeding due to a given generation of ancestors is found after two generations and thereafter remains almost constant (ROBERTSON, 1961). In breeding populations, however, the loss of gene diversity (i.e., group coancestry) already appears in the initial generation of the breeding populations due to the selection, and the gene diversity decreases steadily over generations if there is equal contribution among parents (*Figure 3*).

Conclusions

This study has demonstrated that group coancestry can accumulate fast and status number can decrease fast over generation shifts. The fast change is partly due to the large paternal fertility variation in the material used for the study. The gene diversity preserved in the breeding and production populations may partly be utilised to boost the genetic response. It is concluded from our results that:

1. The loss of diversity is inversely proportional to the status number (N_s) in finite populations. For an idealised population, an expected increment of inbreeding in the next generation will be $1/(2N_s)$ that is the probability that uniting gametes carry identical genes.

2. Inbreeding and reduction of diversity in seed crops over generations are largely influenced by the fertility variation of individuals (measured as Ψ) as well as relatedness among genotypes (described as group coancestry).

3. Breeding population sizes with small status effective numbers will not be able to maintain for longer than a few generations without inbreeding, which may become so severe as to cause fertility problems and to hamper selection.

4. The accumulation of group coancestry cannot be effectively prevented by keeping the maternal fertility constant (*i.e.,* by equalising the number of seeds collected from each mother genotype) if the variation in paternal fertility is very large while the variation in maternal fertility is marginal.

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Effect of Storage Conditions and Seed Treatment on Germination of *Cedrus deodara* LOUD. and *C. libani* A. RICH.

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Abstract

The seeds of *Cedrus deodara* and *C. libani* species were stored during the winter at various temperatures. The storage of the seeds (with initial moisture contents of 19.3% and 18.7% respectively) was made in airtight PVC boxes at temperatures of +5 °C, –10 °C and –20 °C, as well as in a basement at fluctuating temperatures of +10 °C/20 °C. Cones were also stored during the winter in a basement inside common linen sacks. The following spring, the effect of the storage temperatures as well as the effect of the seed treatment (cold stratification and soaking in water) on germination percentage and germination value were investigated. Storage in airtight boxes and at temperatures range +5 °C to –10 °C, were effective short-term storage methods for both of the species. It must be pointed out that during storage, the seeds became dormant that was successfully broken by cold stratification at +5 °C±1 °C for 15 days. The common storage conditions (10 °C/20 °C) as well as temperatures lower than –10 °C had a negative effect on germination of both species. The cone storage of *C. libani* during the winter in the basement was the best method of wintering, because the seeds did not become dormant. On the contrary, cone storage of *C. deodara* in the basement (10 °C/20 °C) during winter is not recommended. The soaking of the seeds in water for 3 hours and the cold stratification at +5 °C±1 °C for 15 or 30 days resulted in a higher seed germination value.

Key words: Cedrus deodara, *Cedrus libani*, stratification, germination percentage, germination value, seed storage, water soaking.

Introduction

According to many references, fresh seeds of *Cedrus* are not normally dormant and thus do not require treatment in order to germinate (DIRR and HEUSER, 1987; TAKOS and MEROU, 1995; HARTMANN *et al.,* 1997). However, it is possible for dormancy to develop in some seed lots whose germination, without treatment, can be irregular. In such cases, if the seeds are treated with cold stratification (+4 °C) for 2 months, then they germinate readily in 4 to 7 days (FORTHAM and SPRAKER, 1977; DIRR and HEUSER, 1987).

The main factors that affect seed viability during the storage are moisture content and temperature (BRADBEER, 1988; BONNER, 1990; GORDON, 1992; TAKOS, 1999a). Storage of many species seems to induce dormancy so that further treatment is necessary (WILLEMSEN, 1975). CHANDRA and RAM (1980) referred to dormancy in stored seeds of *C. deodara*, which was broken after stratification for 15 or 30 days at +4.4 °C. The resulting germination percentages were 16% and 45% respectively, whereas the control (untreated) germination percentage was 11%. THAPLIYAL and GUPTA (1980) also found improvement in the germination percentage, in 6 different seed lots of *C. deodara*, after stratification of the seeds for one week. They also noted that stratification for more than one week (up to four weeks) did not result in any statistically significant difference. According to the same researchers, the best results

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