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Genetic Composition of Seed Orchard Crops

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ABSTRACT

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In three Scots pine seed orchards genetic diversity as measured by the expected panmictic heterozygosity was relatively high and similar. Embryos from Östteg and Robertsfors showed less diversity than the parental clones. Differences in allozyme frequencies were found between some orchards in spite of similarities in their clonal composition. Seed grading had much effect on the genetic structure of embryos from Robertsfors. Embryos from heavy seed were more heterozygous than embryos from light seed.

INTRODUCTION

As the use of seed orchard seed for reforestation increases, the problem of its genetic quality and composition raises more and more concern among foresters. Since seed orchards contain restricted numbers of parents as compared to natural populations, a risk of loss of genetic variability in future plantations is emphasized (Bouvarel, 1970; Adams, 1981). The fact that often only certain clones contribute genes to orchard seed compounds this problem (Jonsson et al., 1976; Müller-Starck, 1982). The origin of clones in a seed orchard usually depends on the seed zone in which orchard crops are to be used and thus it often differs between orchards. Differences in clonal composition are also common between orchards serving one particular seed zone. At the same time, little is known to what extent such differences are reflected in the genetic variation between orchards and their progenies. Seed grading by size, weight, etc. is known to improve germination energy and growth of seedlings and to facilitate the use of certain types of sowing equipment (Lestander, 1985; Werner, 1976). Thus this kind of seed management is often applied in some countries. However, knowledge of the genetic implications of seed grading is scarce.

In this study, allozyme markers were employed to analyse genetic variation in three Scots pine (*Pinus sylvestris* L.) seed orchards and their embryo progenies in Sweden. The following questions were addressed: 1) how much genetic

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diversity exists in different orchards; 2) are there differences between orchards in their genetic composition; and 3) does seed grading by weight affect the genetic structure of seed orchard crops?

MATERIAL AND METHODS

Plant material used in this study originated from three seed orchards of Scots pine in northern Sweden, namely Brån, Östteg, and Robertsfors. The number of clones in these orchards was 34, 30 and 51, respectively. The age of clones varied from 14 to 26 years in Brån, from 17 to 28 years in Östteg, and from 18 to 25 years in Robertsfors. Brån and Östteg had entirely different clones, while 40 and 30% of clones in Robertsfors occurred also in Brån and Östteg, respectively. To determine genotypes of the parental clones, seeds were collected from one graft per clone in each orchard. In addition, bulk seed samples were collected in Östteg and Robertsfors. Before analysis, seeds from Robertsfors were separated into two weight fractions, by a method of seed separation described elsewhere (Lestander, 1985). The average 1000-seed weight in the two fractions was 6.4 and 5.4 g, respectively.

A total of 18 allozyme loci were analysed by starch gel electrophoresis: i.e., leucine aminopeptidase (LAP), 2 loci; glutamate-oxaloacetate transaminase (GOT), 2 loci; acid phosphatase (APH), 1 locus; fluorescent esterase (FEST), 1 locus; shikimate dehydrogenase (SDH), 2 loci; glutamate dehydrogenase, 1 locus; 6-phosphogluconate dehydrogenase (6PGD), 2 loci; malate dehydrogenase (MDH), 2 loci; phosphoglucose isomerase (PGI), 2 loci; phosphoglucomutase (PGM), 2 loci; and aconitase (ACO), 1 locus. Electrophoretic methods used and the inheritance of allozymes have been described elsewhere (Rudin, 1975, 1977; Rudin and Ekberg, 1978; Yazdani and Rudin, 1982; Szmidt, 1984; Szmidt and Yazdani, 1984). Clone genotypes were determined by analysis of 7–12 haploid macrogametophytes. Genotypes of progenies from Östteg and Robertsfors were assessed in embryos.

The observed and the expected heterozygosities were computed from diploid clone and embryo data. Estimates of fixation indices were calculated based on the total observed and the total expected number of heterozygotes according to Curie-Cohen (1982). The genetic distance was computed following Nei (1975).

RESULTS

Of the 18 allozyme loci analysed in this study, 14 were polymorphic in all populations examined. Estimates of genetic diversity and distance were based on all 18 loci analysed, while only 14 polymorphic loci were used for analysis of the genetic structure in fractionated embryos from Robertsfors orchard.

As measured by the average expected heterozygosity (H_e) over 18 loci the

TABLE 1

Observed (H_o) and expected (H_e) heterozygosity, and sample size, N in Scots pine seed orchards and their embryos

Population	H_o	H_e	N
Brån clones	0.288	0.278	34
Östteg clones	0.260	0.261	51
Östteg embryos	0.227	0.235	188
Robertsfors clones	0.287	0.286	30
Robertsfors embryos	0.248	0.260	156

amount of genetic variation was relatively high and similar in seed orchards investigated, ranging from 0.261 to 0.286 (Table 1). The lowest H_e was found in Östteg, which had the highest number of clones. The genetic diversity in embryos from Östteg and Robertsfors was lower than in the parental clones. The same was true for the observed heterozygosity (H_o) in embryos from these orchards.

Genetic distance between Brån and Östteg was only 0.006 though they had entirely different clonal composition. Robertsfors differed more than Brån ($D=0.074$) and Östteg ($D=0.073$) though it shared 40 and 30% of clones, respectively, with these two orchards. There was also, relatively, much difference between embryos from Östteg and Robertsfors ($D=0.142$). Genetic distance between the parental clones and embryos was relatively small, 0.006 to 0.013, for Robertsfors and Östteg, respectively.

Table 2 gives estimates of the expected (H_e) and observed (H_o) heterozygosities and fixation indices (F) in embryos from fractionated Robertsfors seeds. Although there was much variation among loci with respect to the H_e, H_o and F values, some characteristic trends were found. As indicated by fixation values at most loci, there was a trend towards heterozygote excess in embryos from heavy seeds, while homozygote excess was present in embryos from light seeds. This was reflected by the negative average fixation index in heavy seeds as opposed to relatively high and positive average F value in light seeds. In addition, the average observed heterozygosity was higher in embryos from heavy seeds than in embryos from the light seed fraction.

DISCUSSION

Introduction of electrophoretic techniques to forest genetic research has enabled more direct studies of genetic variation and structure in forest trees. As pointed out by Adams (1981), high levels of genetic diversity are essential for maximizing opportunities for selection in the future and minimizing inbreeding. Thus, monitoring genetic diversity in seed orchards can give val-

TABLE 2

Genetic structure at 14 polymorphic loci in Robertsfors clones (C) and embryos from heavy (E-1) and light (E-2) seeds

Locus	Population	N	H_o	H_e	F
LAP-A	E-1	74	0.054	0.053	−0.027
	E-2	82	0.110	0.127	0.123
LAP-B	E-1	74	0.149	0.140	−0.080
	E-2	82	0.146	0.180	0.169
GOT-B	E-1	72	0.597	0.497	−0.213
	E-2	78	0.436	0.539	0.175
APH	E-1	57	0.228	0.207	−0.129
	E-2	77	0.156	0.192	0.303
FEST	E-1	68	0.324	0.375	0.123
	E-2	82	0.390	0.509	0.274
SDH-A	E-1	68	0.456	0.480	0.104
	E-2	82	0.585	0.574	−0.035
SDH-B	E-1	68	0.074	0.071	−0.038
	E-2	82	0.110	0.125	0.123
GDH	E-1	66	0.591	0.481	−0.230
	E-2	82	0.512	0.450	−0.139
6-PGD-A	E-1	72	0.444	0.508	0.086
	E-2	78	0.410	0.500	0.179
6-PGD-B	E-1	72	0.653	0.484	−0.350
	E-2	82	0.402	0.469	0.126
MDH-A	E-1	74	0.135	0.149	0.093
	E-2	82	0.183	0.166	−0.101
MDH-B	E-1	74	0.432	0.426	−0.035
	E-2	81	0.395	0.445	0.111
PGM-A	E-1	74	0.097	0.142	0.311
	E-2	82	0.073	0.071	−0.038
ACO	E-1	21	0.333	0.291	−0.199
	E-2	56	0.357	0.432	0.153
Average	E-1	74	0.326	0.307	−0.042
	E-2	82	0.305	0.341	0.102

N - sample size; H_o - observed heterozygosity; H_e - expected heterozygosity; F - fixation index.

uable information about the genetic base that would be available for advanced generation breeding. Earlier allozyme studies in seed orchards indicated that in spite of restricted clone number, an appreciable amount of genetic variation is usually present among clones (Adams, 1981; Szmidt and Muona, 1985).

Results of the present study give further evidence of relatively high levels of diversity for Scots pine seed orchards in northern Sweden. The orchards differed considerably in clone number and origin; however, their expected panmictic heterozygosities were quite similar. Somewhat surprisingly, lowest diversity was found in Östteg though this orchard had more clones than the other two orchards examined. It thus appears possible that differences in clone number and origin need not have much effect on the levels of genetic diversity in seed orchards measured by isozyme polymorphism. Similar analysis of genetic variation was made in embryos from Östteg and Robertsfors. In both cases, there was slight but distinct decrease of the expected and the observed heterozygosity in embryos as compared with the parental clones. This indicates that only a part of the parental variation is contributed to orchard seed. It must be mentioned here that a more detailed analysis of single-locus data (results not presented in this report) revealed that some embryos at Östteg and Robertsfors received pollen from non-orchard sources. This is in agreement with earlier observations in Robertsfors which indicated that contamination with foreign pollen may be as high as 37% in this orchard (Nagasaka and Szmidt, 1985). It is possible that foreign pollen could also affect estimates of genetic diversity in embryos and that actual loss of parental variation was still greater.

Seed grading is known to improve germination and to facilitate better management of regeneration material (see Lestander, 1985, for a review on this subject). However, as shown by Lindgren (1982) and Lestander (1985), this can also change clone representation in particular seed fractions. A similar conclusion was reached by Friedman and Adams (1982) who studied seeds from *Pinus taeda* orchards. Results from the present study indicate that seed grading by weight may produce changes in genotypic structure of the fractionated crops. It was found that embryos from heavy seeds were more heterozygous than embryos from light seeds, and even contained a slight heterozygote excess; to the contrary, light seeds had a homozygote excess. Since a detailed analysis of genetic structure in embryos was not made in this study, it would be premature to speculate about the factors which could be responsible for this result. Nevertheless, it appears possible that besides deleterious effects such as reduction of clone representation, seed grading may also have positive genetic consequences such as the removal of excessive homozygosity from orchard crops.

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REFERENCES

Adams, W.T., 1981. Population genetics and gene conservation in Pacific Northwest conifers. In: G.G.E. Scudder and J.L. Reveal (Editors), Evolution Today. Proc. Second International Congress of Systematic and Evolutionary Biology, July 1980, Vancouver, B.C. Hunt Institute for Botanical Documentation, Pittsburg, PA, pp.: 401-415.

Bouvarel, P., 1970. The conservation of gene resources in forest trees. In: O.H. Frankel and E. Bennett (Editors), Genetic Resources in Plants; Their Exploration and Conservation. Blackwell, Oxford, pp. 523-530.

Curie-Cohen, M., 1982. Estimates of inbreeding in a natural population: a comparison of sampling properties. Genetics, 100: 339-347.

Friedman, S.T. and Adams, W.T., 1982. Genetic efficiency in loblolly pine seed orchards. In: Proc. 16th Southern Forest Tree Improvement Conference, 27-28 May, 1981, Blacksburg, VA. Virginia Polytechnic Institute and State University, Publ. 38, pp. 213-220.

Jonsson, A., Ekberg, I. and Eriksson, G., 1976. Flowering in seed orchard of *Pinus sylvestris* L. Stud. For. Suec., 135: 38 pp.

Lestander, T., 1985. Fraktionering av frö från barrträd (Grading conifer seeds). Sver. Skogsvårdsför. Tidskr., 1-85: 25-33.

Lindgren, D., 1982. Fractionation of seed orchard seeds by weight does have genetic implications. Silva Fenn., 16: 156-160.

Müller-Starck, G., 1982. Reproductive systems in conifer seed orchards. I. Mating probabilities in a seed orchard of *Pinus sylvestris* L. Silvae Genet., 31: 188-197.

Nagasaka, K. and Szmidt, A.E., 1985. Multilocus analysis of external pollen contamination of a Scots pine (*Pinus sylvestris* L.) seed orchard. Lect. Notes Biomath., 60: 134-138.

Nei, M., 1975. Molecular population genetics and evolution. In: A. Neuberger and E.L. Tatum (Editors), Frontiers of Biology (Vol. 40). North-Holland, Amsterdam, pp. 127-173.

Rudin, D., 1975. Inheritance of glutamate-oxalate-transaminases (GOT) from needles and endosperms of *P. sylvestris*. Hereditas, 80: 296-300.

Rudin, D., 1977. Leucine-amino-peptidases (LAP) from needles and macrogametophytes of *P. sylvestris* L. Inheritance of allozymes. Hereditas, 85: 219-226.

Rudin, D. and Ekberg, I., 1978. Linkage studies in *Pinus sylvestris* L. using macrogametophyte allozymes. Silvae Genet., 27: 1-12.

Szmidt, A.E., 1984. Genetic of Scots pine (*Pinus sylvestris* L.) domestication by means of isozyme analysis. Ph.D. Dissertation, The Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, Umeå, 186 pp.

Szmidt, A.E. and Muona, O., 1985. Genetic effects of Scots pine (*Pinus sylvestris* L.) domestication. Lect. Notes Biomath., 60: 241-252.

Szmidt, A.E. and Yazdani, R., 1984. Electrophoretic studies of genetic polymorphism of shikimate and 6-phosphogluconate dehydrogenases in Scots pine (*Pinus sylvestris* L.). Arbor. Kórnickie, 29: 63-72.

Werner, M., 1976. Effekter på plantutbyte, höjdtillväxt och höjdvariation vid fraktionering av ett granfröparti (Effects of spruce seed grading on plant percent, height growth and variation). Inf. Skogsföräd., 8, 1975/76, Institute för skogsförbättring, Ekebo, 4 pp.

Yazdani, R., and Rudin, D., 1982. Inheritance of fluorescent esterase and β-glucosidase in haploid and diploid tissues of *Pinus sylvestris* L. Hereditas, 96: 191-194.