http://www.pjbs.org



ISSN 1028-8880

# Pakistan Journal of Biological Sciences



# Fertility Variation and Status Number in Clonal Seed Orchards of Pinus sylvestris

Nebi Bilir and Halime Temirağa Faculty of Forestry, Suleyman Demirel University, Isparta, TR-32260, Turkey

**Abstract:** The present study was carried out to evaluate fertility variation, status number and gene diversity based on strobili productions in two clonal seed orchards of Scots pine (*Pinus sylvestris* L.). There were large differences among clones for the female and male strobili productions in the orchards. Positive and significant ( $p \le 0.05$ ) correlations were found between female and male strobili production (r = 0.76, 0.55). Female fertility variation (1.03, 1.07) was larger than male fertility variation (1.02, 1.03) in the orchards. The status numbers estimated based on the total fertility were very high (97 and 98% of census numbers). The large fertility variation could be balanced by different treatments such as mixing seed equally from clones or genetinc thinning.

**Key words:** Scots pine, graft, gene diversity, coancestry

## INTRODUCTION

Seed orchards are one of the important seed sources for plantation forestry and a link between present and future forests as gene conservation areas. They are becoming gradually more importance for conversion of unproductive forest to productive forest because of its improved seeds. For instance, while the species occupy roughly 750000 ha, of which about 475000 ha are considered to be productive forests, only 10% of the seed demand in the species was supplied (Cengiz, 2003) in from 111 ha of seed orchards in Turkey. New seed orchards have been established according to the "National Tree Breeding and Seed Production Programme" of Turkey (Koski and Antola, 1993). One of the factors that have impact on seed production is the variation in fertility, among and within clones in the orchards (Prescher et al., 2007). Gene diversity in orchard crops is a function of fertility variation and pollen contamination. Many studies were conducted on fertility variation in forest tree species (Bila, 2000; Kang, 2001; Kang and Lindgren, 1998; Bilir et al., 2002, 2004; Varghese et al., 2006) for different purposes such as genetic management of populations (Bila, 2000), breeding (El-Kassaby, 1995; Griffin, 1982; Dutkuner et al., 2008) and gene conservation programs (Kang et al., 2003). Gene diversity is regarded to be of importance for the sustainability of forest ecosystems. Gene diversity in seed orchard crops can play an important role in coping with climate change as it may make the seed crop more robust and more adapted to a range of environments. Gene diversity is one of the relevant considerations when establishing and managing seed orchards (Prescher et al., 2007) and for gene diversity estimates information on fertility variations is

needed (Lindgren and Mullin, 1998). Many studies were carried out on female and male fertility in seed orchards (Kjaer, 1996; Kang and Lindgren, 1998; Nikkanen and Ruotsalainen, 2000). To monitor and determine the genetic diversity of seed crops, some genetic parameters such as genetic relatedness, inbreeding and gene diversity should be calculated.

The present study aimed to evaluate fertility variation among orchard parents and its effect on status number and gene diversity and to contribute breeding programme of the species.

## MATERIALS AND METHODS

The study was carried out in two clonal seed orchards of Scots pine (*Pinus sylvestris*). The seed orchards established with originated from plus trees, which were selected from a specific seed stand. The seed orchards were established at Mengen, Bolu (40°56′N, 32°13′E, 850 m) at spacing 7×7 m consisting of 30 clones and 1034 grafts in 1988 and at Taskopru, Kastamonu (41°36′N, 35°05′E, 1500 m) at 6×6 m consisting of 30 clones and 1987 grafts in 1995.

Female and male strobili data were collected on three grafts, chosen at random, from each of twenty two clones of Mengen orchard and twenty five clones of Taskopru orchard in the spring of 2005.

**Fertility variation and status number:** In this study, fertility variation is estimated based on the variation in strobilus production among individuals in the populations. The fertility variation for female  $(\psi_t)$  and male  $(\psi_m)$  were estimated by Kang and Lindgren (1999) as:

$$\psi_f = N \sum_{i=1}^N \left( \frac{f_i}{\sum f_i} \right)^2 = C V_f^2 + 1 \tag{1a} \label{eq:psi_f}$$

$$\psi_{\rm m} = N \sum_{\rm i=1}^{N} \left( \frac{m_{\rm i}}{\sum m_{\rm i}} \right)^{\! 2} = {\rm CV}_{\rm m}^{2} + 1 \eqno(1b)$$

where, N is the census number,  $f_i$  is the number of female strobili of the ith individual,  $m_i$  is the number of male strobili of the ith individual and  $\mathrm{CV}_f$  and  $\mathrm{CV}_m$  are the coefficients of variation in female and male strobilus production among individuals.

Total fertility variation  $(\Psi)$  was calculated by Kang and Lindgren (1999) as :

$$\begin{split} \Psi &= N \sum_{i=l}^{N} \left( \frac{0.5f_{i}}{\sum f_{i}} + \frac{0.5m_{i}}{\sum m_{i}} \right)^{2} \\ &= 0.25 \left[ N \sum_{i=l}^{N} \frac{f_{i}^{2}}{\sum f_{i}} + N \sum_{i=l}^{N} \frac{m_{i}^{2}}{\sum m_{i}} + N \sum_{i=l}^{N} \frac{2m_{i}f_{i}}{\sum m_{i} * \sum f_{i}} \right] \end{split} \tag{2}$$

where, N is the census number, f<sub>i</sub> and m<sub>i</sub> are the numbers of female and male strobili of the ith individual, respectively.

The status numbers of female  $(N_{s(t)})$  and male parents  $(N_{s(m)})$  are calculated based on female fertility variation  $(\psi_t)$ , male fertility variation  $(\psi_m)$  and the census number (N) by Lindgren *et al.* (1996) as:

$$N_{s(f)} = N/\psi_f \tag{3a}$$

$$N_{s(m)} = N/\psi_m \tag{3b}$$

Status number on total fertility was calculated following Kang (2001) and as:

$$N_{s} = \frac{4N}{[\Psi_{f} + \Psi_{m} + 2 + 2r\sqrt{(\Psi_{f} - 1)(\Psi_{m} - 1)}]}$$
(4)

where,  $\psi_f$  and  $\psi_m$  are the fertility variations of female and male parents, respectively and r is the correlation

coefficient between female and male strobilus production. Relative status number  $(N_r)$  was calculated as  $N_r = N_s/N$  (Lindgren *et al.*, 1996).

#### RESULTS AND DISCUSSION

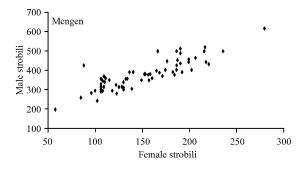
**Female and male strobilus production:** The average, coefficient of variation, ranges of female and male strobilus production and phenotypic correlation coefficients between female and male strobili were given in Table 1.

Clonal averages of female and male strobili were 150.4 and 371 in Mengen orchard and 179.8 and 238.5 in Taskopru orchard, respectively (Table 1). There were large variations in strobilus production among clones in the orchards (Fig. 1).

The variations of female strobili production were higher than that of male strobili. Large clonal differences in female and male fertility have been reported in seed orchards (Kjaer, 1996; Kang and Lindgren, 1998; Nikkanen and Ruotsalainen, 2000). Differences in gamete contribution among clones could be genetic (Eriksson et al., 1973), environmental (Hedegart, 1976) and management of orchard (Zobel and Talbert, 1984). Thus, could also be other factors such as age and the associated tree size (Bilir et al., 2006), or seed orchard locations and years (Lindgren et al., 1977). For instance, averages of female and male strobili production were reported 201.4 and 531.3 at 16 years (Bilir et al., 2002) and 254.3 and 724.3 at 19 years (Dutkuner et al., 2008) in a Turkish Scots pine seed orchard.

Broad-sense heritability was very low (<0.16) in Turkish scots pine seed orchards. It means that to create desirable characters in seed orchard trees, there ought to be a large potential of management, which affects "the environment" (Bilir *et al.*, 2006).

It is reported that fertility variation is higher when seed orchards are young (Kang and Lindgren, 1999; Bila, 2000). In the present study, data on strobilus



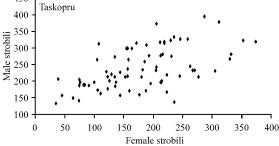


Fig. 1: Relation between female and male strobili production in the orchards

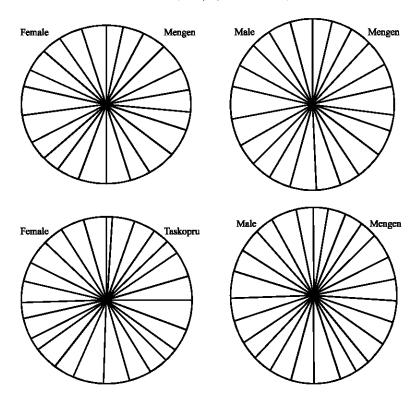


Fig. 2: Parental gamete contribution of clones in the orchards

Table 1: Average, coefficient of variation (CV), range and correlation (r) in female and male strobilus production in the studied seed orchards

|                    | Seed of chards |         |          |         |  |
|--------------------|----------------|---------|----------|---------|--|
|                    | Mengen         |         | Taskopru |         |  |
|                    |                |         |          |         |  |
| Statistics         | Female         | Male    | Female   | Male    |  |
| Average            | 150.4          | 371     | 179.8    | 238.5   |  |
| CV                 | 0.179          | 0.134   | 0.257    | 0.158   |  |
| Range (clonal)     | 111-196        | 253-485 | 55-267   | 160-308 |  |
| Range (individual) | 58-279         | 35-610  | 35-374   | 133-394 |  |
| r <sup>a</sup>     | 0.763          |         | 0.552    |         |  |

\*Phenotypic correlation coefficient between clonal female and male strobilus production

production were collected from only one year. Kang (2001) reported that clonal fertility was fluctuated over many years in conifer seed orchards.

The most abundant quartile of clones in Mengen orchard produced 28.3% of total female and 32.1%, while they were 31% and 24% in Taskopru orchard, respectively (Fig. 1).

Positive and significant ( $p \le 0.05$ ) correlations were found between female and male strobilus production in both of the orchards (Table 1, Fig. 2).

This result is well in accordance with the results from seed orchards of different forest tree species by Bilir *et al.* (2002), Keskin (1999), Jonsson *et al.* (1976), Bhumibhamon (1978), Burczyk and Chalupka (1997), Nikkanen and Ruotsalainen (2000) and Varghese *et al.* 

(2000). However, negative genetic correlations between female and male flowering were reported in different forest tree species by Schultz (1971) and Savolainen *et al.*, (1993). The correlation coefficient between female and male fertility should thus be considered when the total fertility is estimated (Kang, 2001; Kang and El-Kassaby, 2002).

Coefficients of variation of females were higher than those of males in the seed orchards (Table 1). Large differences in the production of female and male strobili among clones were found in seed orchards by Kang (2001) and by Keskin (1999).

**Fertility variation and status number:** Female  $(\psi_f)$ , male  $(\psi_m)$  and total  $(\Psi)$  fertility variation, status number  $(N_s)$  and relative status number  $(N_r)$  were presented in Table 2. Female fertility variation  $(\psi_t)$  was higher than male fertility variation  $(\psi_m)$  in the orchards (Table 2). Similar results were also reported by Bilir *et al.* (2002) in a clonal seed orchard of Turkish Scots pine, while it was opposite to Dutkuner *et al.* (2008).

The estimated status numbers were very high in the orchards (Table 2). They were 21.3 (97% of census number) for female parents, 21.6 (98%) for male parents and 21.5 (98%) for total fertility in Mengen orchard and 23.5 (94%), 24.4 (98%) and 24.2 (97%) in Taskopru

Table 2: Fertility variation  $(\iota_r \text{ and } \iota_m)$ , status number  $(N_{s(n)} \text{ and } N_{s(m)})$  and relative status number  $(N_{t(n)} \text{ and } N_{t(m)})$  in gametic gene pool for female and male parents

| Temare                                  | Seed orchar | Seed orchards |             |               |  |  |  |
|---|-------------|---------------|-------------|---------------|--|--|--|
|   | Mengen (22  | 2)*           | Taskopru (2 | Taskopru (25) |  |  |  |
| Parameters                              | Female      | Male          | Female      | Male          |  |  |  |
| ι <sub>f</sub> and ι <sub>m</sub> **    | 1.03        | 1.02          | 1.07        | 1.03          |  |  |  |
| N <sub>s(f)</sub> and N <sub>s(m)</sub> | 21.3        | 21.60         | 23.5        | 24.40         |  |  |  |
| N <sub>r(f)</sub> and N <sub>r(m)</sub> | 0.97        | 0.98          | 0.94        | 0.98          |  |  |  |

<sup>\*</sup>Census number of clones (N) in parentheses, \*\*F and m represent female and male, respectively

Table 3: Fertility variation  $(\Psi)$ , status number  $(N_s)$ , relative status number  $(N_t)$  and relative gene diversity (GD) in total gametic gene pool in the studied seed orchards

|                     | Seed orchards |              |  |  |
|---------------------|---------------|--------------|--|--|
| Parameters          | Mengen (22)*  | Taskopru(25) |  |  |
| Ψ                   | 1.023         | 1.031        |  |  |
| $N_s$               | 21.500        | 24.200       |  |  |
| $N_r$               | 0.980         | 0.97         |  |  |
| $GD (= 1-1/(2*N_s)$ | 0.977         | 0.979        |  |  |

Census number (N) in parentheses

orchard, respectively (Table 2, 3). Similar results were also reported by Bilir *et al.* (2002) and Dutkuner *et al.* (2008) in a Turkish Scots pine seed orchard.

Gene Diversity (GD) in seed orchard crop was maintained high (0.977 and 0.979) as seen in Table 3. Maximum gene diversity of seed orchards crop can only be attained when all parents contribute equally to the gamete gene pool (Kang, 2001). The relation between Gene Diversity (GD) and fertility variation ( $\Psi$ ) and census number of clones (N) can be showed as: GD = 1-0.5  $\Psi$ /N. When used many clones in establishment of seed orchard, fertility variation among clones and within clone could be high (e.g., CV).

Relative effective number of clones and the coefficient of variation among clones for ramet numbers were estimated 0.76 and 55.6% in Mengen (Anonymous, 2012) orchard and 0.95 and 23% in Taskopru orchard based on ramet numbers of clones by Bilir and Ulusan (2007). Based on genetic information from progeny tests, new seed orchards will be established in the species according the "Turkish Breeding Programme". Parental trees are selected initially according to their phenotypes for traits such as vigour, form, wood quality or other desired characteristics, which include general adaptability.

## CONCLUSION

Genetic information (e.g., genetic value, fertility) could also be used as a criterion of selection for seed production in seed orchards. Equal seed harvest or mixing seed equally from clones may improve the fertility variation and thus status number or balance by different

management treatments such as pruning. It could be also balanced by equal or similar number of grafts each clone.

## ACKNOWLEDGMENTS

Authors acknowledge administrative support from The Research Directorate of Forest Tree Seeds and Tree Breeding of Turkey. We thank to anonymous reviewers who made valuable comments which helped to improve the manuscript.

## REFERENCES

Anonymous, 2012. Website of the research directorate of forest tree seeds and tree breeding of Turkey. http://www.ortohum.gov.tr/english/.

Bhumibhamon, S., 1978. Studies on Scots Pine Seed Orchards in Finland with Special Emphasis on the Genetic Composition of the Seed. Metsantutkimuslaitos, Finland, ISBN-13: 9789514003479, Pages: 118.

Bila, A.D., 2000. Fertility variation and its effects on gene diversity in forest tree populations. Ph.D. Thesis, Swedish University of Agricultural Science, Umea, Sweden.

Bilir, N., K.S. Kang and H. Ozturk, 2002. Fertility variation and gene diversity in clonal seed orchards of *Pinus brutia*, *Pinus nigra* and *Pinus sylvestris* in Turkey. Silvae Genet., 51: 112-115.

Bilir, N., K.S. Kang, D. Zang and D. Lindgren, 2004. Fertility variation and status number between a base population and a seed orchard of *Pinus brutia*. Silvae Genetica, 53: 161-163.

Bilir, N., F. Prescher, S. Ayan and D. Lindgren, 2006. Growth characters and number of strobili in clonal seed orchards of *Pinus sylvestris*. Euphytica, 152: 293-301.

Bilir, N. and M.D. Ulusan, 2007. Seed orchards and seed collection stands of Scots pine in Turkey. Proceedings of the Seed Orchard Conference, September 26-28, 2007, Umea, Sweden, pp. 25-36.

Burczyk, J. and W. Chalupka, 1997. Flowering and cone production variability and its effect on parental balance in a Scots pine clonal seed orchard. Ann. Sci. For., 54: 129-144.

Cengiz, N., 2003. Inventory of supply and demand of seed for Turkey. B.18.0.AGM.0.04.02.781/81/443, Ankara, Turkey. http://www.ogm.gov.tr/.

Dutkuner, I., N. Bilir and M.D. Ulusan, 2008. Influence of growth on reproductive traits and its effect on fertility and gene diversity in a clonal seed orchard of Scots pine, *Pinus sylvestris* L. J. Environ. Biol., 29: 349-352.

- El-Kassaby, YA., 1995. Evaluation of the treeimprovement delivery system: Factors affecting genetic potential. Tree Physiol., 15: 545-550.
- Eriksson, G., A. Jonsson and D. Lindgren, 1973. Flowering in a clonal trial of *Picea abies* Karst. Studia Forestalia Suecica No. 110, pp: 4-45. http://epsilon.slu.se/studia/SFS110.pdf
- Griffin, A.R., 1982. Clonal variation in radiata pine seed orchards. I: Some flowering, cone and seed production traits. Aust. For. Res., 12: 295-302.
- Hedegart, T., 1976. Breeding System, Variation and Genetic Improvement of Teak (*Tectona grandis* Linn. f.). In: Tropical Trees, Burley, J. and B.T. Styles (Eds.). Academic Press, London, UK., pp. 102-123.
- Jonsson, A., I. Ekberg and G. Eriksson, 1976. Flowering in a seed orchard of *Pinus sylvestris* L. Studia Forestalia Sueccica No. 135, Stockholm, Sweden. http://pub. epsilon.slu.se/5612/1/SFS135.pdf
- Kang, K.S. and D. Lindgren, 1998. Fertility variation and its effect on the relatedness of seeds in *Pinus densiflora*, *Pinus thunbergii* and *Pinus koraiensis* clonal seed orchards. Silvae Genetica, 47: 196-201.
- Kang, K.S. and D. Lindgren, 1999. Fertility variation among clones of Korean pine (*Pinus koraiensis* S. et Z.) and its implications on seed orchard management. For. Genet., 6: 191-200.
- Kang, K.S. and Y.A. El-Kassaby, 2002. Considerations of correlated fertility between genders on genetic diversity: *Pinus densiflora* seed orchard as a model. Theor. Applied Genet., 105: 1183-1189.
- Kang, K.S., 2001. Genetic gain and gene diversity of seed orchard crops. Ph.D. Thesis, Swedish University of Agricultural Science, Umea, Sweden.
- Kang, K.S., A.D. Bila, A.M. Harju and D. Lindgren, 2003. Estimation of fertility variation in forest tree populations. Forestry, 76: 329-344.
- Keskin, S., 1999. Clonal variation in flowering and cone characteristics in a *Pinus brutia* seed orchard. Technical Bulletin of Southwest Anatolia Forest Research Institute No. 9, Antalya, Turkey, pp. 1-96.
- Kjaer, E.D., 1996. Estimation of effective population number in a *Picea abies* (Karst.) seed orchard based on flower assessment. Scand. J. For. Res., 11: 111-121.

- Koski, V. and J. Antola, 1993. National tree breeding and seed production programme for Turkey 1994-2003. pp: 52
- Lindgren, D. and T.J. Mullin, 1998. Relatedness and status number in seed orchard crops. Can. J. For. Res., 28: 276-283.
- Lindgren, D., L. Gea and P. Jefferson, 1996. Loss of genetic diversity monitored by status number. Silvae Genet., 45: 52-59.
- Lindgren, K., I. Ekberg and G. Eriksson, 1977. External factors influencing female flowering in *Picea abies* (L.) Karst. Studia Forestalia Sueccica No. 142, Stockholm, Sweden. http://pub.epsilon.slu.se/5514/1/ SFS142.pdf
- Nikkanen, T. and S. Ruotsalainen, 2000. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. Silva Fennica, 34: 205-222.
- Prescher, F., D. Lindgren, C. Almqvist, J. Kroon, T. Lestander and T. Mullin, 2007. Female fertility variation in mature *Pinus sylvestris* clonal seed orchards. Scand. J. For. Res., 22: 280-289.
- Savolainen, O., K. Karkkainen, A. Harju, T. Nikkanen and M. Rusanen, 1993. Fertility variation in *Pinus sylvestris*: A test of sexual allocation theory. Am. J. Bot., 80: 1016-1020.
- Schultz, R.P., 1971. Stimulation of flower and seed production in a young slash pine orchard. USDA Forest Service Research Paper SE-91, USA., pp: 1-10.
- Varghese, M., A. Nicodemus, B. Nagarajan and D. Lindgren, 2006. Impact of fertility variation on gene diversity and drift in two clonal seed orchards of teak (*Tectona grandis* Linn. f.). New For., 31: 497-512.
- Varghese, M., A. Nicodemus, B. Nagarajan, B. Sidappa, K.R. Sasidharan, S.S.R. Bennet and K. Subramianian, 2000. Seedling Seed Orchards for Breeding Tropical Trees. Scrool Press, Coimbatore, India, ISBN: 81-900346-1, Pages: 126.
- Zobel, B. and T. Talbert, 1984. Applied Forest Tree Improvement. 1st Edn. John Wiley and Sons, New York, USA, ISBN 0-471-09682-2, Pages: 505.