portance for survival of the ovules during winter period and subsequent fertilization of the egg cell in the second developmental season. It is evident therefore that this feature of pollinated ovules reflects very sensitively the hybridological status of the species under investigation and that it may be utilized effectively in the evolutionary study of the hard pines as recommended earlier by Mc WILLIAM (1959).

A close relationship between *P. nigra* and *P. thunbergii* at the biochemical level has been demonstrated in our comparative study concerning the serological properties of the pollen grains (KORMUTAK and LANAKOVA, (1988) and the restriction profiles of the cloroplast DNA (KORMUTAK et al., in press). All the findings obtained so far correlate positively with the established compatibility of *P. nigra x P. thunbergii* combination. PRUS-GLOWACKI et al. (1985) in their serotaxonomical investigation of pines revealed the presence of seed antigens common for both the above species. The authors share opinion that except for the continuing speciation of pines and considerable intraspecific variability, it is irregular hybridization which complicates the systematics of the genus Pinus. The last mentioned aspect is fully applicable to the group Lariciones as well. Of no less strange than compatible hybridological status of geographically distinct species of *P. nigra* x *P. thunbergii* and *P. nigra* x *P. tabulaeformis* is also a strong reproductive isolation of the species combinations of *P. mugo* x *P. nigra* (KORMUTAK and LANAKOVA, 1988), *P. nigra* x *P. sylvestris* and reciprocal (VIDAKOVIČ and JURKOVIČ-BEVILACQUA, 1970; VIDAKOVIČ and BORZAN, 1973) as well a sa negligible crossability between *P. sylvestris* and *P. mugo* (DOBRINOV and JAGDZIDIS, 1971; NEET-SARQUEDA et al., 1988) the evolution of all of which was to a large degree bound to common territory of Europe.

References

CRITCHFIELD, W. B.: Interspecific hybridization in Pinus: A summary review. Proc. 14th meeting Can. tree improv. assoc., Part 2. Ottawa 1975: 99—105 (1975). — DOBRINOV, J. and JAGDZIDIS, G.: Za spontannite chybridy meždu belija bor i kleka u nas. Gorsko Stopanstvo 11: 28—30 (1971). — DUFFIELD, J. W.: Relationships and species hybridization in the genus Pinus. Z. Forstgenetic 1: 93—100 (1952). — GARRETT, W. P.: Species hybridization in the genus Pinus. Forest Service Research Paper NE-436, Forest Service, U. S. Dept. of Agriculture, Northeastern Forest Exper. Station, Broomall, PA 19008, 17 pp. (1979). — JOHNSON, A. G.: Spontaneous white pine hybrids. Jour. Arnold Arboretum XXXIII: 179—185 (1952). — KORMUTAK, A. and LANAKOVA, M.: Biochemistry of reproductive organs and hybridological relationships of selected pine species (*Pinus* sp.). Acta dendrobiologica, VEDA Bratislava, 119 pp. (1988). — KRIBEL, H. B.: The timing of the incompatibility reaction in interspecific crosses of *Pinus strobus* L.. Proc. IUFRO Congress, Munich 1967, Part III, Sect. 22: 77—87 (1967). — LUNDKVIST, K.: Genetic differentiation within and among Swedish populations of Norway spruce (*Picea abies*). Proc. Confr. Biochem. Genetic Forest Trees. (Ed. D. RUDIN). Umeå, Sweden, 1978: 113—117 (1978). — LUX, A.: Rýchla metóda polotenkých rezov z rastlinných objektov. Biológia 36 (9): 753—757 (1981). — MCWILLIAM, J. R.: Interspecific incompatibility in Pinus. Amer. J. Bot. 46: 425—433 (1959). — MIROV, N. T.: The genus Pinus. The Ronald Press Company, New York. 602 pp. (1967). — NEET-SARQUEDA, A., PLUMETTAZ, G. and BÉCHOLEY, I.: Mise en évidence de l'hybridization introgressive entre *Pinus sylvestris* L. et *Pinus uncinata* Dc. and Valais (Suisse) par deux méthodes multivariées. Bot. helvetica 98 (2): 161—169 (1988). — PILGER, R.: Gymnospermae. In: ENGLER und PRANTL: Die natürlichen Pflanzenfamilien. 2. Aufl. Leipzig. 302 pp. (1926). — PRUS-GLOWACKI, W., SZWEYKOWSKI, J. and NOWAK, R.: Serotaxonomical investigation of the European pine species. Silvae Genetica 34 (4—5): 162—170 (1985). — RUDIN, D. and RASMUSON, B.: Genetic variation in esterase from needles of *Pinus sylvestris L.*. Hereditas 73: 89—98 (1973). — SAX, K. and SAX, H. J.: Chromosome number and morphology in the conifers. Jour. Arnold Arboretum 14: 357—375 (1933). — SHAW, G. R.: The genus Pinus. Arnold Arboretum, Publ. No. 5, 96 pp. (1914). — SIMAK, M.: Some problems concerning X-ray contrast methods used in forestry seed testing and research. Res. Notes No. 55. Dept. Reforestration, Royal College of Forestry, Stockholm 1—14 (1974). — TANG-SHUI LIU: A monograph of the genus Abies. Taiwan National University, Taiwan. 608 pp. (1971). — VIDAKOVIČ, M. and BORZAN, Ž.: Contribution to the investigation of incompatibility by crossing Scots pine and European black pine. Proc. IUFRO Symp. Genet. Scots pine, Warszaw-Kórnik 1—19 (1973). — VIDAKOVIČ, M. and JURKOVIČ-BEVILACQUA, B.: Observations on the ovule development following cross-pollination between Austrian and Scots pines using irradiated and non-irradiated pollen. Proc. Sexual Reprod. For. Trees, Vol. 3, Varparanta. 8 pp. (1970).

Clonal Variation in Heat Tolerance and Heat Shock Protein Expression in Black Spruce

By S. J. COLOMBO[1]), M. L. COLCLOUGH[2]), V. R. TIMMER[3]), and E. BLUMWALD[2])

(Received 20th June 1991)

Summary

One-year-old *Picea mariana* rooted cuttings representing 9 families and a total of 24 clones were used to assess variability in heat tolerance and, for 2 clones, differences in heat shock protein (HSP) synthesis. Different clones of the same family exposed to a temperature of 47 °C for 30 minutes varied widely in damage, indicating a large component of single tree variability in the factors contributing to heat tolerance. The predominance of clones from family 347 possessing above-average heat tolerance suggested that tolerance is, in part, also heritable within families. Clone 86—109 was both more heat tolerant and possessed higher constitutive levels of HSP compared to clone 347—36. Levels of HSP synthesis offer promise as a means of molecular selection for tree breeding and clonal propagation. Rapid screening for heat tolerance of large numbers of individuals is possible by means of direct hot water immersion of shoots.

Key words: Picea, stress tolerance, rooted cutting, heat shock.

[1]) Ontario Forest Research Institute, Ontario Ministry of Natural Resources, PO Box 969, 1235 Queen St. E., Sault Ste. Marie, Ontario, Canada P6A 5N5.

[2]) Centre for Plant Biotechnology, Department of Botany, 25 Wilcocks St., Toronto, Ontario, Canada M5S 3B2.

[3]) Faculty of Forestry, University of Toronto, 33 Wilcocks St., Toronto, Ontario, Canada M5S 3BS2.

Introduction

For black spruce (*Picea mariana* MILL. B. S. P.), a boreal temperate zone species with a vast range spanning the

northern section of North America from the Pacific to the Atlantic Oceans, heat tolerance is not commonly considered an important trait for tree improvement. However, high temperatures can cause damage during nursery stock production, stock handling, and after planting. During nursery stock production, damaging high temperatures can occur to germinants and seedling foliage in greenhouses (Colombo, 1990) and after transplanting in nursery beds (Korstian and Fetherolf, 1921). The heat tolerance of foliage is also of importance during nursery stock handling and shipping, particularly when stock is packaged in bags or boxes when direct exposure of the foliage to high temperatures can occur (Kauppi, 1984). High soil surface temperatures after planting can be a significant factor causing injury or mortality in trees up to age 10 years, even in northerly latitudes (Baker, 1929; Helgerson, 1990; Smith, 1951; Vaartaja, 1949). In addition, if predictions concerning global environmental warming are accurate, the ability of native trees to tolerate high temperatures, thermotolerance, may be an important attribute for tree improvement purposes, as has been the case for various agricultural species (Marsh et al., 1985; Reynolds and Ewing, 1989; Wang and Nguyen, 1989).

Tree improvement of black spruce is facilitated by the fact that shoots from juvenile individuals of this species can be propagated relatively easily using rooted cuttings. This has led to the implementation of operational clonal propagation programs for black spruce, including the Moonbeam Clonal Forestry Centre located near Kapuskasing in northern Ontario, Canada. Clonal propagation offers many potential advantages in comparison to seed-based propagation, chief among which are the ability to propagate large numbers of genetically superior individuals in a relatively short period of time for direct transmission of desirable traits into tree planting operations (Libby and Rauter, 1984). However, in order to derive this advantage it is necessary to identify traits conferring genetic superiority while individuals are still young enough to possess high juvenile rooting ability. The early identification of individuals possessing genetically controlled traits is also important in tree improvement programs based on family selection. One means of such early testing is the assessment of seedling stress tolerance. This paper presents a technique for screening large numbers of individuals for heat tolerance, which can be used both in clonal screening and selective tree breeding programs.

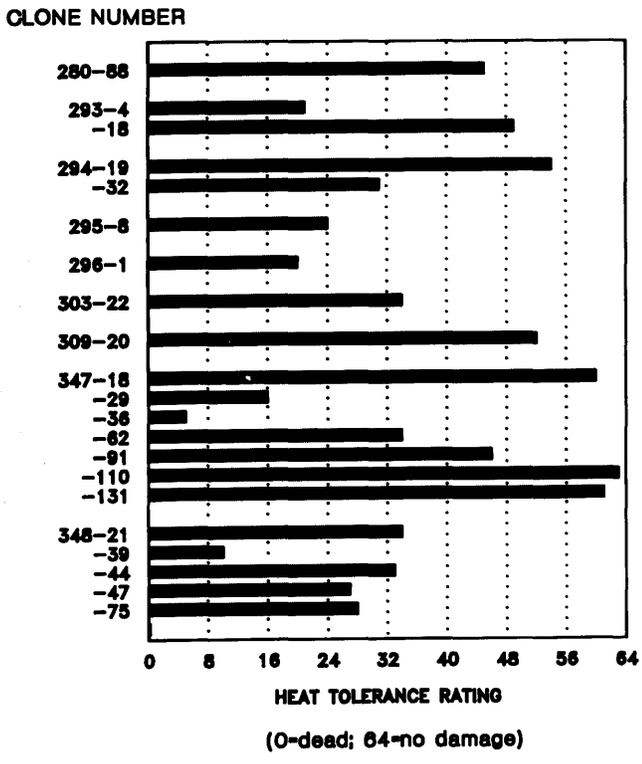

Figure 1. — Clonal variation in heat tolerance. Black spruce rooted cuttings were exposed to 47°C for 30 minutes as described in Materials and Methods. Damage to the terminal 5 cm of each rooted cutting was rated according to a qualitative scale 4 weeks after heat exposure, where: 0 = entire terminal 5 cm of shoot dead; 1 = stem alive, needles all killed, no buds growing; 2 = stem alive, needles all killed, buds growing on the lower half of the terminal 5 cm of stem; 3 = buds growing, damage restricted to the apical meristem and the needles immediately surrounding it; and, 4 = no damage to the terminal 5 cm of the shoot.

Exposure of black spruce to high, non-damaging temperatures increases subsequent thermotolerance (Koppenaal *et al.*, 1990; Colclough *et al.*, 1992). The induction of thermotolerance is dependant on temperature and duration of exposure, whereby seedlings exposed to 38 °C for 3 hours are able to survive subsequent high-temperature stresses which would otherwise be lethal. The development of thermotolerance is characterized by increased synthesis of Heat Shock Proteins (HSPs) (Colclough *et al.*, 1992). During exposure to high temperatures the synthesis of several heat shock proteins is induced and/or enhanced, and HSPs have been implicated in the development of thermotolerance in black spruce (Colclough et al., 1992).

The purpose of this investigation is to (1) establish the sensitivity of black spruce clones to heat stress and (2) to assess the relationship between clonal differences in heat tolerance and heat shock protein synthesis.

Materials and Methods

Heat tolerance of black spruce rooted cutting shoots was evaluated using plant material originating from seedlings produced from controlled pollination between plus trees. On September 7, 1989, rooted cuttings from 9 families representing 24 clones were exposed to a temperature of 47 °C for 30 minutes to test heat tolerance levels. Fourteen clones came from two full-sib families, 9 from family 347 and 5 from family 348. Clones from families 347 and 348 were selected on the basis of the results of field screening trials (unpublished) conducted by the Northern Region of the Ontario Ministry of Natural Resources near the towns of Cochrane, Hearst and Kapuskasing: clones with the best and poorest survival were selected for screening based on the hypothesis that field survival rates were at least partly due to post-planting heat stress. An additional 10 clones from 7 families other than families 347 and 348 were tested to compare inter-family differences in heat tolerance. Sixteen rooted cuttings per clone were tested. A list of all clones tested is shown in *figure 1.*

Heat tolerance was tested between 12:30 pm and 3:30 pm. High temperature exposure was accomplished by inverting the trays of transplanted rooted cuttings and plunging the shoots to within approximately 1 cm of the container surface in a 47 °C ± 0.1 °C circulating hot water bath for 30 minutes. Exposure for 30 minutes to 47 °C was chosen on the basis of other investigations (Colombo and Timmer, 1992), which indicated this to be an exposure level sensitive to differences in heat tolerance. In order to ensure that no injury resulted from immersion in water apart from the

effects of elevated temperature, black spruce shoots were immersed in room temperature water for up to 3 hours; this immersion caused no damage (COLOMBO and TIMMER, 1992). SHIRLEY (1936) similarly found that 5 hours immersion of conifer seedling shoots in room temperature water caused no damage.

Following removal from the water bath the trays of rooted cuttings were placed in a greenhouse for four weeks to allow the full expression of damage symptoms to take place. At the end of four weeks, damage to the terminal 5 cm of each rooted cutting was rated according to a qualitative scale, where: 0 = entire terminal 5 cm of shoot dead; 1 = stem alive, needles all killed, no buds growing; 2 = stem alive, needles all killed, buds growing on the lower half of the terminal 5 cm of stem; 3 = buds growing, damage restricted to the apical meristem and the needles immediately surrounding it; and, 4 = no damage to the terminal 5 cm of the shoot. A clonal heat tolerance rating was calculated by summing the code values for the 16 rooted cuttings per clone, with clonal heat tolerance being greater the higher the value. This non-destructive assessment of heat tolerance was used to preserve the surviving rooted cuttings for use in other trials.

A second test of clonal heat tolerance was conducted in June, 1990, using clones 86—109 and 347—36, in order to compare heat tolerance to the ability of these clones to produce heat shock proteins (HSPs). These clones were selected on the basis of their wide variation in heat tolerance in a prior investigation (COLOMBO, unpublished). In this test, 15 rooted cuttings per clone were immersed in 47 °C water for 75 minutes. This longer duration of exposure than was used in the first test was necessary, as plants in June had initiated buds, which is known to increase heat tolerance (KOPPENAAL and COLOMBO, 1988), whereas those tested the previous September had not initiated buds. No difference in interpretation due to rooted cuttings being bud-initiated is expected, as species differences in heat tolerance do not usually interact with season (BANNISTER, 1970; BANNISTER and SMITH, 1983). Damage in the June test was expressed as the proportional dry weight of damaged to non-damaged shoot, four weeks after heat exposure.

For heat shock protein extraction and immunological detection of heat shock proteins, 25 rooted cuttings each of clones 86—109 and 347—36 were removed from the growing medium, the roots rinsed with tap water and placed in aerated plastic containers with 500 ml of complete nutrient solution (20—20—20) and incubated overnight under normal growth conditions. The following day, rooted cuttings were exposed to 126 °C or 48 °C air temperature in a growth chamber for 1 hour (relative humidity 70% to 80% and light intensity 220 μmol m^{-2} sec^{-1} to 240 μmol m^{-2} sec^{-1}), and soluble and membrane-bound proteins were extracted as described before (COLCLOUGH et al., 1992). This temperature-duration combination was chosen based on previous investigations of heat shock protein expression (COLCLOUGH et al., 1992). Nuclear membrane proteins were collected by centrifugation of homogenate at 200 x g for 15 minutes. Proteins were separated electrophoretically using SDS-PAGE on 10% discontinuous linear acrylamide gels and thereafter transferred onto nitrocellulose by electroblotting under constant voltage (30V) for 21 hours. Immunological detection of HSPs was done using a mouse monoclonal antibody, specific for the constitutive HSP_{73} and inducible HSP_{72} from Hela cells (StressGen Biotech. Corp., Sidney, B. C., Canada).

Results and Discussion

Wide differences in heat tolerance occurred between black spruce clones exposed to high temperature (*Fig. 1*). Three clones (347—18, 347—110 and 347—131) had clonal heat tolerance ratings of 60 or greater, indicating virtually no damage. In contrast, three clones (347—36 and 348—39) had heat tolerance ratings below 16, indicating severe damage or mortality of almost all rooted cuttings tested.

Clones belonging to family 347 tended to be more heat tolerant than clones from family 348 (*Fig. 1*). This may indicate that genetic differences at the family level play a role in determining heat tolerance. Since variation in heat tolerance between clones from the same family was also great (eg. clones 347—110 and 347—36), it suggests that both inter- and intra-family factors play important roles in determining heat tolerance. Heat tolerance and metabolism under elevated temperatures have been found to be heritable traits in a wide range of plant species (LARSON, 1989; MARSH et al., 1985; MARTINEAU et al., 1979; NEALES, 1968; REYNOLDS and EWING, 1989; WANG and NGUYEN, 1989).

One of the principal difficulties in the development of clonal forestry programs for large scale reforestation is the identification of superior clones. The results of the clonal screening of heat tolerance described in this trial offers one means of selecting clones. High temperature is an environmental constraint upon plant development for which a clonal selection program may prove an effective means of improving stress tolerance. Similar screening protocols to those described here could easily be adapted to the selection of parents for controlled pollination to increase the heat tolerance of offspring in a tree breeding program. In this trial, it appears that family variability in heat tolerance of black spruce exists, which would enable a breeding program based on this trait to yield improvements in heat tolerance of the offspring. This screening protocol can also be applied to individuals from general collection seed sources. This has been done

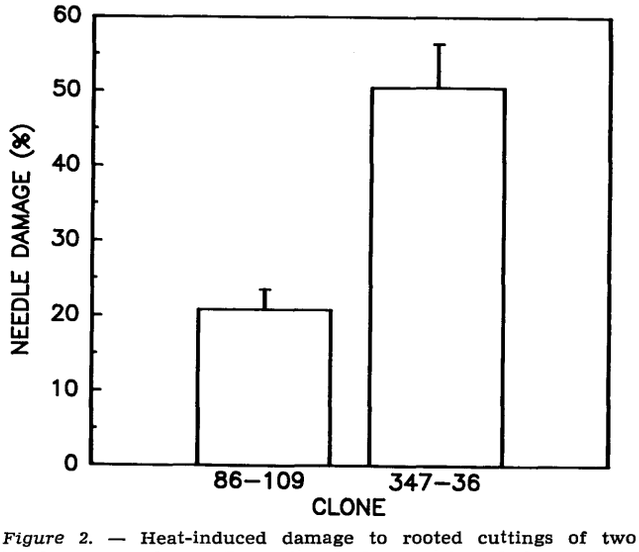

Figure 2. — Heat-induced damage to rooted cuttings of two black spruce clones. Proportional dry weight of damaged to non-damaged shoots of clones 86—109 and 347—36, 4 weeks after immersion in 47°C water for 75 minutes.

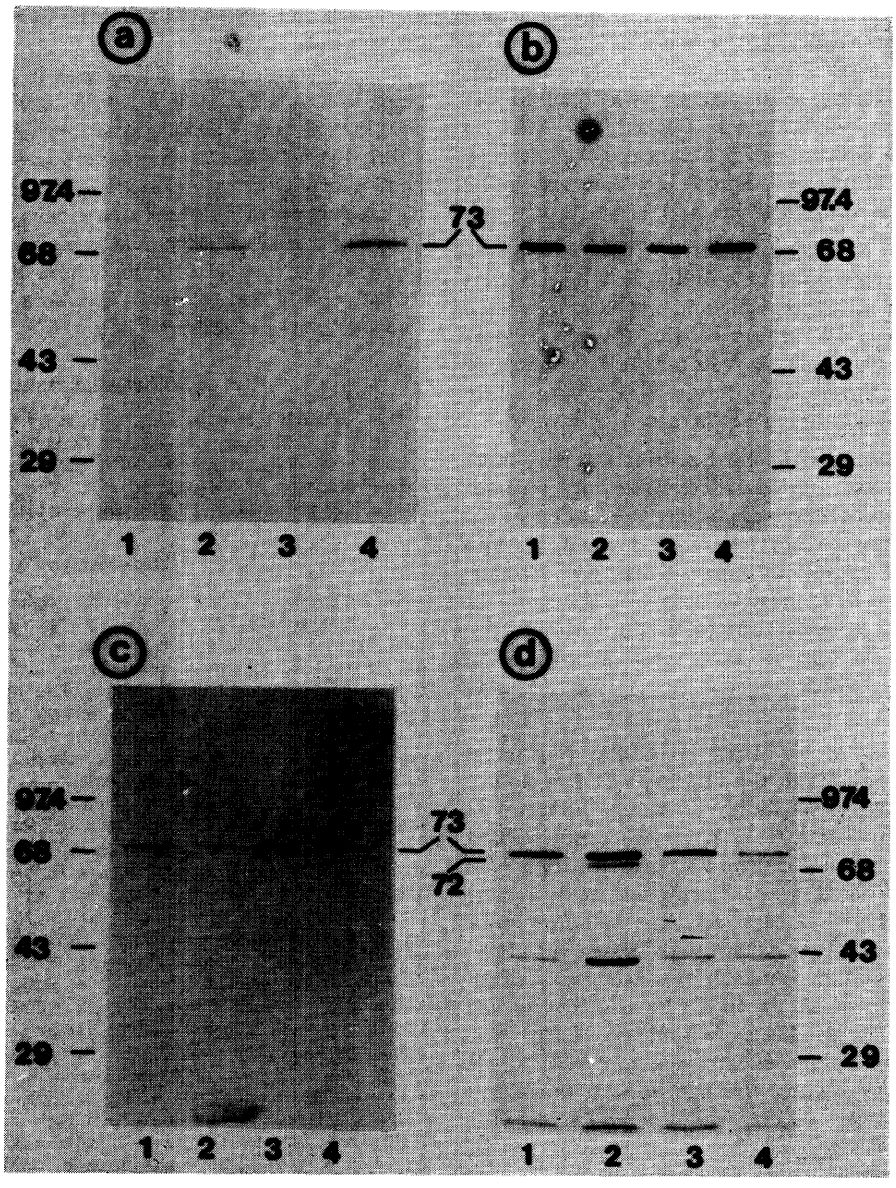

Figure 3. — Immunological detection of heat shock proteins. Black spruce rooted cuttings were exposed at 126°C or 48°C in a growth chamber for 1 hour and soluble and membrane-bound protein fractions extracted and prepared for SDS-PAGE as described in Materials and Methods. Proteins were locaded onto 10% acrylamide linear gels. Following electrophoresis, proteins were transferred onto nitrocellulose by electroblotting. After blotting, heat shock proteins were deteced by immunoreaction with a monoclonal antibody for the HSP72/73 from HeLa cells, as described in Materials and Methods.

(a) root nuclear proteins. 3 ug protein/lane.
Lanes: (1) CL86—109 at 26° C, (2) CL86-109 at 48° C, (3) CL347—36 at 26° C, (4) CL—347—36 at 48° C.
(b) root mitochondrial proteins. 8 ug protein/lane.
Lanes: (1) CL86—109 at 26° C, (2) CL86—109 at 48° C, (3) CL347—36 at 26° C, (4) CL—347—36 at 48° C.
(c) root microsomal proteins. 8ug protein/lane.
Lanes: (1) CL86—109 at 26° C, (2) CL86—109 at 48° C, (3) CL347—36 at 26° C, (4) CL—347—36 at 48° C.
(d) root soluble proteins. 8 ug protein/lane.
Lanes: (1) CL86—109 at 26° C, (2) CL86—109 at 48° C, (3) CL347—36 at 26° C, (4) CL—347—36 at 48° C.

(Colombo, unpublished) using trays of general seed source collection container-grown seedlings in which the shoots were immersed in hot water, as was done in this trial for clonal stock. Individuals selected using this approach can then either be propagated clonally or used in conventional tree improvement programs. This assumes that a high level of heat tolerance and HSP expression is a superior attribute, with no unforseen negative side effects. Field testing in support of this assumption is necessary to safely apply this protocol in a tree improvement program.

In order to assess the possible role of HSPs in the tolerance of heat stress, two clones found in an earlier trial to differ in levels of heat tolerance (Colombo, unpublished) were evaluated to compare the relative levels of heat tolerance and HSP expression. As shown in *figure 2*, clone 86—109 was significantly more heat tolerant than clone 347—36. These same clones were exposed to heat shock conditions (sudden exposure to 48 °C), and the constitutive and inducible levels of $HSP_{72/73}$ were studied by immunological detection using a monoclonal antibody for the $HSP_{72/73}$ from human HeLa cells (Colclough *et al.*, 1992). We have found that this antibody specifically recognized $HSP_{72/73}$ from black spruce seedlings, and that the enhanced synthesis of $HSP_{72/73}$ was correlated with heat-tolerance (Colclough et al., 1992).

Both heat-resistant and heat-sensitive black spruce clones displayed constitutive synthesis of $HSP_{72/73}$ at 26 °C (*Fig. 3*). $HSP_{72/73}$ was observed in soluble, nuclear, mitochondrial, and microsomal protein fractions. In the soluble protein fractions of both clones, the $HSP_{72/73}$ antibody not only recognized two polypeptides of molecular masses 72 kD and 73 kD, but also recognized two polypeptides of molecular masses 42 kD and 23 kD.

The exposure of the heat-sensitive clone 347—36 to 48 °C resulted in a decrease of soluble $HSP_{72/73}$ and an increase in nuclear, mitochondrial and microsomal membrane-bound $HSP_{72/73}$. In comparison, exposure of heat-resistant clone 86—109 to 48 °C resulted in a decrease of $HSP_{72/73}$ in membrane-bound protein fractions, while soluble $HSP_{72/73}$ was significantly increased (*Figure 3*).

Despite their designation as heat shock proteins, most black spruce HSPs are expressed constitutively without the need for high temperature exposure (Colclough *et al.*, 1992), thus suggesting a role for these proteins in the normal physiological activity of black spruce cells. Proteins of the HSP_{70} family have been shown to participate in the stabilization of protein substrates that are in the process of maturation and in posttranslational protein assembly and translocation (Chirico et al., 1988; Deshaies *et al.*, 1988).

Recently, Beckmann *et al.* (1990) suggested that most newly synthesized proteins in the cell interact transiently with cytosolic $HSP_{72/73}$. Through such an interaction, new proteins are maintained in a stable conformation until translation is completed. The new protein may then begin to fold into its final conformation with a concomitant release of $HSP_{72/73}$. Morever, the HSP_{70} group of proteins have autoregulatory capabilities (Tilly *et al.*, 1982) and a positive correlation between the accumulation of HSP_{70} and repression of its synthesis has been observed (DiDomenico et al., 1982; Colclough *et al.*, 1992). The concentration of free versus substrate-bound $HSP_{72/73}$ is tightly regulated; thus, when the equilibrium is shifted toward substrate-bound forms, the reduction of free $HSP_{72/73}$ will induce $HSP_{72/73}$ synthesis (Beckmann *et al.*, 1990). In the current trial, heat-shock induced a marked increase in cytosolic $HSP_{72/73}$ in a heat-tolerant black spruce clone. In a heat-sensitive clone, cytosolic $HSP_{72/73}$ decreased, although membrane-bound $HSP_{72/73}$ increased in mitochondrial, microsomal and nuclear fractions. These results suggest that although newly synthesized proteins were able to complete their insertion in the membranes, they were unable to release the substrate-bound $HSP_{72/73}$. Moreover, our results also suggest that the regulatory mechanisms of HSP synthesis were affected in heat-sensitive clones, since the increase in the bound form of $HSP_{72/73}$ failed to induce $HSP_{72/73}$ synthesis.

Clonal differences in HSP synthesis and heat tolerance suggest that these traits are under genetic control in black spruce. Genetic variation in the level of HSP synthesis has been reported in higher plants. In sorghum, genetic differences in high temperature sensitivity are correlated with variations in the capacity for HSP synthesis (Ougham and Stoddart, 1986). In wheat, a correlation was identified between the synthesis of specific low molecular weight heat shock proteins and the degree of thermotolerance expressed following exposure to high temperatures (Krishnan *et al.*, 1989). In the case of black spruce in the present study, clonal differences in heat tolerance and HSP synthesis may similarly reflect genetic control over these traits.

In conclusion, large differences in clonal heat tolerance suggest the existence of both inter- and intra-family control of the physiological attributes controlling the ability to tolerate high temperatures. Relative levels of heat tolerance were associated with the constitutive levels of heat shock proteins in two clones, and with the ability to produce HSPs in response to high temperature exposure. Using the protocols described here heat tolerance can be tested rapidly and for large numbers of individuals in tree improvement programs.

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References

Baker, F. S.: Effect of excessively high temperatures on coniferous reproduction. J. For. **27**: 949—975 (1929). — Bannister, P.: The annual course of drought and heat resistance in heath plants from an oceanic environment. Flora **159**: 105—123 (1970). — Bannister, P. and Smith, P. J. M.: The heat resistance of some New Zealand plants. Flora **173**: 399—414 (1983). — Beckmann, R. P., Mizzen, L. A. and Welch, W. J.: Interaction of HSP 70 with newly synthesized proteins: Implication for protein folding and assembly. Science **248**: 850—853 (1990). — Chirico, W. J., Waters, M. G. and Blobel, G.: 70K heat shock related proteins stimulate protein translocation into microsomes. Nature **332**: 805—810 (1988). — Colclough, M. L., Colombo, S. J., Koppenaal, R. S. and Blumwald, E.: High temperature stress in black spruce seedlings. Thermotolerance and the synthesis of heat shock proteins. (in preparation) (1992). — Colombo, S. J.: Heat tolerance of tree seedlings and its importance to nursery managers. Proc., 1990 Prairie Tree Nursery Assoc. Meeting, Pineland Provincial Nursery, Sept. 10 to 12, 1990. Manitoba Dept. Nat. Resources (1990). — Colombo, S. J. and Timmer, V. R.: Critical levels of exposure resulting in direct and indirect high temperature damage to black spruce. Tree Physiology (in press) (1992). — Deshaies, R. J., Koch, B. D., Werner-Washburne, M., Craig, E. A. and Schekman, R.: A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. Nature **332**: 800—805 (1988). — Didomenico, B. J., Bugaisky, G. E. and Lindquist, S.: The heat shock response is self-regulated at both the transcriptional and postranscriptional levels. Cell **31**: 593—603 (1982). — Helgerson, O. T.: Heat damage in tree seedlings and its prevention. New Forests **3**: 333—358 (1990). — Koppenaal, R. S. and Colombo, S. J.: Heat tolerance of actively growing, bud initiated, and dormant black spruce seedlings. Can. J. For. Res. **18**: 1103—1105 (1988). — Koppenaal,R. S., Colombo, S. J. and Blumwald, E.: Acquired thermotolerance of jack pine, white spruce and black spruce seedlings. Tree Physiology **8**: 83—91 (1990). — Korstian, C. F. and Fetherolf: Control of stem girdle of spruce transplants caused by excessive heat. Phytopathology **11**: 485—490 (1921). — Krishnan, M., Nguyen, H. T. and Burke, J. J.: Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiol **90**: 140—145 (1989). — Larson, D. W.: Differential heat sensitivity amongst four populations of the lichen *Ramalina menziesii* Tayl. New Phytol. **111**: 73—79 (1989). — Libby, W. J. and Rauter, R. M.: Advantages of clonal forestry. Forest. Chron. **60**: 145—149 (1984). — Marsh, L. E., Davis, D. W. and Li, P. H.: Selection and inheritance of heat tolerance in the common bean by use of conductivity. J. Amer. Soc. Hort. Sci. **110**: 680—683 (1985). Martineau, J. R., Williams, J. H. and Specht, J. E.: Temperature tolerance in soybeans. II. Evaluations of segregating populations for membrane thermostability. Crop. Sci. **19**: 79—81 (1979). — Neales, T. F.: Effects of high temperature and genotype on the growth of excised roots of *Arabidopsis thaliana*. Aust. J. Biol. Sci. **21**: 217—223 (1968). — Ougham, H. J. and Stoddart, J. L.: Synthesis of heat shock proteins and acquisition of thermal tolerance in high temperature tolerant and high temperature susceptible lines of sorghum. Plant Sci. **44**: 163—167 (1986). — Reynolds, M. P. and Ewing, E. E.: Heat tolerance in tuber bearing *Solanum* species: A protocal for screening. Amer. Potato J. **66**:

63—74 (1989). —— SHIRLEY, H. L.: Lethal high temperatures for conifers, and the cooling effect of transpiration. J. Agr. Res. 53: 239—258 (1936). —— SMITH, D. M.: The influence of seedbed conditions on the regeneration of eastern white pine. Conn. Age. Exp. Stn. Bull. No. 545, New Haven. 61 p. (1951). —— TILLY, K., MCKITTRICK, N., ZYLICS, M. and GEORGOPOULOS, C.: The dnak protein modulates the heat-shock response of Escherichia coli. Cell 34: 641—646 (1983). —— WANG, W. C. and NGUYEN, H. T.: Thermal stress evaluation of suspension cell cultures in winter wheat. Plant Cell Rep. 8: 108—111 (1989).

Provenance Trial in Eucalyptus grandis and its Implication to Forestry Programmes

By K. N. SUBRAMANIAN, A. K. MANDAL, P. GOVINDARAJ and K. R. SASIDHARAN

Institute of Forest Genetics and Tree Breeding, Coimbatore 641 002, India

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Summary

A provenance trial involving 17 exotic provenances from Australia and one local (Valparai) seed source of *Eucalyptus grandis* was undertaken to identify suitable provenances for a general plantation programme. The study revealed the presence of significant variation among the provenances. The provenances Paluma, Ravenshoe, Gadgara and Herberton were found to be the best. It is suggested that the local provenance of *E. grandis* which has till now been used for plantation forestry in the region should be replaced with the fast-growing provenance, Paluma. This may also be used as the population for the genetic improvement of *E. grandis*, if undertaken.

Key words: Eucalyptus grandis, provenance, variability, correlation.

Introduction

Selection of the best provenances of the best species for a given site or region is necessary for achieving maximum productivity both in plantation forestry and with agroforestry systems. The concept of provenance testing has been well established and demonstrated through nationally or internationally conducted co-ordinated provenance trials. *Eucalyptus grandis*, an exotic species, is being introduced to India for its high value for fuel, pulp, paper and timber and is the most sought-after species for paper production. It is also resistant to "pink disease" and grows well at high altitude in Tamil Nadu and Kerala (SHYAM SUNDAR, 1979). Information on the amount of provenance variation in growth rate and on selection of superior provenance of this species for commercial planting is meagre. A trial was therefore established so that suitable provenances of *Eucalyptus grandis* could be recommended for general and agroforestry plantation programmes.

Materials and Methods

The experimental materials consisted of 17 Australian provenances of *Eucalyptus grandis* received from CSIRO, Australia and one local seed source (*Table 1*). The provenance trial was planted in 1981 at Valparai which is a hill station, and at 1066 metres a. m. s. l. The provenances were planted in a randomized block design with four replications. Sixteen plants were planted in each plot at an espacement of 2 metres x 2 metres. Height and girth were measured annually. At the final assessment at age 9 years, height, clear bole height, girth and diameter at breast height (dbh) and basal area were measured. Growth parameters at the age of 7 and 9 years form the basis of this paper. The data were subjected to analysis of variance using plot means and correlation coefficients between characters were calculated at the plot mean level.

Results

Significant differences among the provenances were shown in the analysis of variance for all the characters under study (*Table 2*).

Provenance means for different characters are presented in *table 3*. All the provenances except S. of Tyalgum, northern N. S. W., Port Stephens and Eungella were superior to the Valparai local seed source for height which ranged from 17.8 m (Port Stephens) to 25.5 m (Paluma) at the age of 7 years. However, at the age of 9 all the Australian provenances were growing better than the Valparai local source. Of the 17 provenances, 11 showed 25% better growth in height than control. Mean height ranged from 26.2 m (Valparei local) to 44.0 m (Paluma). The correlation between heights at the ages of 7 and 9 years was positive and significant (r = 0.7188). Clear bole height measured at the age of 9 years was higher in all Australian provenances than the Valparai local and ranged from 10.3 m (Valparai local) to 26.1 m (Paluma).

12 and 7 provenances respectively had higher girth at the age of 7 and 9 years than the Valparei local. While Paluma showed the highest girth (82.0 cm), the Port Stephens provenance recorded the lowest girth (48.2 cm). The correlation between the girths at 7 and 9 years of age was positive and significant (r = 0.9089).

Mean values for dbh ranged from 13.5 cm (Port Stephens) to 23.6 cm (Paluma). 11 provenances had better diameter growth rate than the local plantation.

11 out of 17 provenances recorded higher mean basal area than the Valparai local and the values ranged from 143.9 cm^2 (Port Stephens) to 446.3 cm^2 (Paluma). 6 provenances showed 50% increased basal area over Valparai local. However, interplot competetion is likely to be exaggerating differences between poor-performing and good-performing provenances.