Preliminary results of hybridization

of some eucalypt species

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I. Introduction

Eucalypts are a group of species that are widely planted in many regions in our country (especially in Central and South Vietnam provinces) to supply paper and particle board raw material, mine-props, constructional timber and firewood. These are also main species planted along the roads in rural areas, along rice-field bunds in the Red River Delta in the North and Mekong River Delta in the South.

Results of research and cultivation in many passing years show that of the eucalypt species introduced in our country only some have fast growth and are of high adaptability, note worthy are Eucalyptus urophylla, E.camaldulensis and E.exserta.

Interspecific crossing between these species aimed at creating hybrids of high productivity and adaptability to concrete sites will contribute to meeting the demand for raw material for industries mine-props, constructional timber, firewood and protection forest planting in some regions in our country in contribution to the implementation of the planting of 3 million of hectares of production and protection forests.

According to Martin (1989) there had been upto 1989 over 20 interspecific hybrid combinations, created in the Eucalyptus genus of which the main group consists of E.grandis and E.urophylla as mother trees. They are E.grandis crossed with E.robusta, E.camaldulensis, E.tereticornis, E.saligna and E.pellita, E.urophylla crossed with E.grandis, E.pellita and E.resinifera. A series of hybrid combinations between E.alba, E.camaldulensis, E.microtheca, E.tereticornis, E.polycarpa and E.torelliana were also created in 1991-1999 period in India (Paramathma, Surendran, 2000).

Natural hybrid between E.camaldulensis and E.robusta that was previously detected in Vietnam (Le Dinh Kha, 1970) also has markedly faster growth as compared with its parental species.

Thus the carrying out of two-way crossing between E.urophylla, E.exserta and E.camaldulensis aimed at creating some hybrid combinations of high productivity will contribute to improved forest plantation productivity, quickly accomplishing the programme for planting new 3 million hectares of production and protection forests in Vietnam.

II. Materials and research methodology

Materials used in the research are some plus trees, 7-10 year-old, selected from best provenances or local provenance of E.urophylla, E.exserta and E.*camaldulensis.* E.urophylla provenances used are those of fast growth such as Lewotobi, Egon and Lembata (Nguyen Duong Tai, 1994, Le Dinh Kha, 1996, 2001, Pham Van Tuan and collaborators, 2000). E.*camaldulensis* (C)
and E.exserta (E) are local provenances suffering no die-back disease. (Sharma, 1994).

Interspecific and intraspecific crossing of the above - mentioned eucalypt species has been implemented by controlled pollination in the 1996-2000 period. Prior to pollination the flowers were made free of their own pollen and covered with control bags. Controls are trees from seedlings with seed supplied by CSIRO (such as $U_{egon}U_{lem}$ and C_{ken}), local provenances (Csx , Usx , Esx) and progeny of graft trees from plus trees as well as provenance Kien Tai (C_{KT}) of E.camaldulensis and hybrid combination GU (E.grandis x E.urophylla) exchanged with CSIRO.

Trials for comparison between hybrid combinations and their parental species and with the planting stock used in production were established on main site types:

-Red River alluvial soil in Thuy Phuong (Ha Noi)
-Feralitic soil on denuded hills generated from sand stone in Ba Vi (Ha Tay).
-Feralitic soil on denuded hills generated from mica-schist in Dong Ha (Quang Tri).
-Seasonal Water-submerged sulphat acid soil in Kien Giang.

The trials were planted with 3 x 2m spacing, 6-10 tree blocks, 3-6 replicates. 3 kg of decomposed farm manure and 200g of thermophosphate were applied to each planted tree. In Ba Vi alone 500kg of powder C_aCO_3 was applied per hectare. The data collected are diameter breast high ($D_{1..3}$), total tree

height (H). Stem volume over-bark (V) is calculated by formula $V= P.D^2/4.H.f$ (f is stem form coefficient estimated as 0.5).

Study on pulp potential was carried out at Paper and Cellulose Institute. Study on leaf morphology and anatomy and propagation by meristem tissue culture was carried out at the Research Centre for Forest tree Improvement.
Data processing was done by software program GENSTAT 5.3 (William Matheson, 1994).

III. Research Results and Discussion

1. Some researches on phenology, pollen and pollination.

1.1. Special features of phenology of eucalypt species used in hybridization.

Study on special phenological features of eucalypt species involved in hybridization at Ba Vi in 1995-1996 shows that the flowering period of E.camaldulensis was from Feb. 25th to June, 10th. The flowering period of E.exserta was from May 15th to July 10th. Of these two species there were 25 days of overlapping flowering (May 15 - June 10). Thus some natural interspecific hybrids can be created. The flowering of E.urophylla is from August 5th to Oct. 30th. To have hybrids between E.urophylla and other two species controlled pollination must be done (artificial hybridization).

1.2.Identifying viability of the pollen and storage period.

After being dried by silica gel the pollen was stored in conditions of room temperature (20-30^{0}C), ordinary refrigerator and deep freeze (-30^{0}C).

Being stored in room temperature condition the germination ability of the pollen is almost entirely lost after 1 month (remaining 13-16%) in ordinary refrigerator, it remains 45-50% after 1 year; in deep-freeze the germination rate remains 50-70% after 4 years. The germination rate of E.camaldulensis pollen after 4 years is highest, i.e. 78.9% ; those of E.urophylla and E.exserta are 50.7 and 30% respectively.

1.3.Effect of pollination mode on fruiting ratio and other indices.

Experiments with and without using control bag prior to flower opening with eucalypt species at Ba Vi and Chem show that without control bag (free pollination) the fruiting rate of E.exserta and E.urophylla is 75.7-86.1% ; with control bag no seed is formed. As regards E.camaldulensis with or without control bag no or very few seed is formed. This means that although the flower of eucalypts is bisexual, it is entirely sterile with self pollination inside the control bag.
Weight of 1,000 seed produced by various hybrids varies little or much depending on concrete parental species involved in the hybridization. Besides the role of the female parent there appears also xemia seed.

2. Characteristics of F1 hybrids.

Many hybrid combinations have been created in hybridization. In 1997 there were only 3 first hybrid combinations created at Ba Vi and Chem i.e. U_{29} C_3, $U_{29}E_2$ and E_2C_3. In 1998, 17 hybrid combinations were used in trials: In 1999 42 hybrid combinations were used in trials. In 2000 more tens of hybrid combination were created and were not used in trial.

2.1.Leaf morphological and anatomical indices.

Study on some morphological and anatomical typical features of some two-way hybrid combinations such as U_{29} E_1, E_1 U_{29}, U_{17} C_1, C_1 U_{17} and their parents E_1, C_1, U_{29} and U_{17} shows that the length of leaf petiole of the hybrids might greater (such as U_{17} C_1 and C_1 U_{17}) or intermediate between parental species (U_{29} E_1 and E_1 U_{29}). On the other hand the length, width and area of the leaf of hybrid combinations are all greater or intermediate while leaf index (length/width) is always intermediate between the parental species.

An utmost marked expression of the intermediate nature is the number of air apertures on upper leaf surface of the hybrid combinations U C, UE. E.urophylla has no air aperture on the upper leaf surface. E.camaldulensis and E.exserta have air apertures on upper leaf surface and the hybrid combinations UE and UC all have air apertures on upper leaf surface. The number of air apertures is intermediate between the parental species.

2.2.Size of branches and branch angle.

Size of the branch is determined by actual measurement and by relative value (ratio between branch diameter close to the stem and stem diameter at the branching point. Hybrid has its stem diameter at the branching point greater than the parental species but the relative size of its branch is smaller than the parental species thus it has higher value in tree selection than the parental species although its branching angle is intermediate or a little smaller than the parental species.

2.3.Variation in peroxidase in hybrid combinations and the parental species.

Leaf sample analysis by different peroxidase for hybrid combinations U_{29} E_1, E_1 U_{29}, U_{29} E_2, E_2 U_{29} and their parental species U_{29}, E_1 and E_2 by electrophorus method of Moran and Bell (1983) shows that E_1 U_{29}, E_2 U_{29} hybrids all receive Rf0.14 and Rf0. 18 bands from the parent trees ; U_{29} E_1, U_{29} E_2 receive only Rf 0,18 band from U and E ; while Rf.0.10 band is new and alien to their parents.

This means that in two-way cross-breeding if U_{29} is male parent, the hybrid receives both $Rf_{0.N}$ and $Rf_{0.18}$ bands of the two parents. If U_{29} is used as female parent, the hybrid shows a new band ($Rf_{0.10}$), non-existent with its parents. In other words, two-way crossing changes the biochemistry of the hybrid.

3. Growth of eucalypt hybrid combinations and the parental species.

3.1. Trial of eucalypt hybrid combinations in 1997.

Measurement of growth of hybrids at 9-months of age at Cam Quy (Ha Tay) and Chem (Ha Noi) shows that at Chem (alluvial soil of Red River) $U_{29}C_3$ is a hybrid combination of fastest growth and next comes E_2C_3, and last are parental species $U_{29}E_2$ (Le Dinh Kha, Nguyen Viet Cuong, 1998).

At Cam Quy in conditions of denuded hill soil poor in nutrients and deficient water in Autumn-Winter season, at 3 years of age the hybrid combination $U_{29}E_2$ shows best heterosis in growth and next comes the hybrid combination $U_{29}C_2$. Ranking last is still E_2 C_3. Growth of all hybrid combinations is faster than that of parent species.

3.2.Trial of eucalypt hybrid combinations in 1998.

Trial to compare growth of eucalypt hybrid combinations in 1998 was carried out on Red River alluvial soil (at Thuy Phuong) and on denuded hill soil with thin soil layer, poor in nutrients (at Ba Vi).

Diagram 2. Stem volume of eucalypt hybrid combinations at Thuy Phuong and Ba Vi at 3 years of age.

Research results in the two first years show that hybrids at Thuy Phuong have fast growth and at the same arial marked heterosis as compared with the parents. (Le Dinh Kha, Nguyen Viet Cuong, 2000 a.b). At 3 years of age although survival of planted trees on both sites is all high (85-100%) but on alluvial soil, eucalypt hybrids and the parents all have marked faster growth than on denuded hill soil.

Average stem volume in the trial at Thuy Phuong is 74.3dm^3/tree and at Ba Vi it is 25.2dm^3/tree, On the other hand, superiority in growth (heterosis) of hybrids as compared with the parents is obviously shown at Thuy Phuong while at Ba Vi although heterosis is also shown but it is far less. Finally, while at Thuy Phuong hybrid combinations UC have fastest growth, at Ba Vi hybrid combination UE have fastest growth. Hybrid combinations EC always have slow growth on both sites.

3.3.Trial of eucalypt hybrid combinations in 1999.

In 1999 at Cam Quy 42 hybrid combinations were planted in comparison with 12 parents (progeny of plus trees or planting stock used in production). In Kien Giang 14 eucalypt hybrid combinations were planted in comparison with 3 sources of planting stock used in production (U_{sx}, C_{sx}, E_{sx}) and the source of E.camaldulensis of Kien Tai company (C_{KT}). In Dong Ha 9 hybrid combinations were planted in comparison with the sources used in production and with PN_2, PN_{14} of Phu Ninh Paper Raw Material Centre as well as in comparison with U_6 imported by Central forest planting stock company from China.
Results of trial after one year and a half at BaVi show that of the 42 eucalypt hybrid combinations used, 17 have faster growth than the fastest growth used in production U_{sx}. 5 hybrid combinations have stem

volume 15-62,9% greater than U_{sx}. They are U_{15} C_4, GU, U_{30} U_{23}, U_{30} E_5 and U_{29} E_2. Trial in Kien Giang shows that the survival of E.exserta and E.urophylla is low (38.8 -50%). Hybrids $U_{29}E_1$ and U_{29} U_{26} all have low survival (55.6%). These hybrids are not suitable for the water - submerged sulphate acid soil here. Of the 14 hybrid combinations, 5 combinations (U_{15} C_4, E_1 U_{29}, U_{29} E_2, E_2 U_{29}, and U_{16} C_2 have stem volume 74-111% and 40.1-65.6% greater than that of C_{sx} and C_{KT} respectively. Trial at Dong Ha lasts only over one year. Preliminary results show that PN_2, PN_{14} have growth poorer than U_{sx} Hyblid combinations U_{15} C_4, U_{29} E_2, U_2 U_{29}, U_{29} E_1 and E_1 U_{29} have their height greater than that of U_6 as well as of other control formulae. However, the trials established in 1999 have lasted too little arial and the above preliminary remarks are by no means certain.

4. Ability of disease and insect pest resistance of some eucalypt hybrid combinations.

In the trial area established in 1997 there already occurred a leaf bug of psilidae family, homoptera order. In 9-month old plantations, 3 eucalypt parent species all are damaged at level 55.3-66.6%. The damage level of hybrid combinations (UE, UC and EC) is only 10.5-23.3%. The level of attack (expressed by points - O point: no attack, 5: greatest attack) of the hybrid combinations is 0.10 - 0.25 while in parent species it is 1.35-1.62.

In trial established in 1998 at Cam Quy there appears leaf margin necrosis, leaf blight and bark canker. Leaf margin necrosis appears in C ken and Ct due to Pestalotiopsis with ratio of damaged trees 50% and 42.4% respectively and level of attack 2.8 and 4.3 (5 -point scale). Hybrid combinations and other species suffer no attack. Leaf blight and die back caused by Cylindrocladium and Pestalotiopsis (Sharma 1994) appear in all experimental formulae at various levels. Ratio of the damaged trees of the parent species is 50.3-90%, level of attack 2.6-4.3 while in hybrid combinations UE, EU, UC, UU the ratio in only 2.5%-16.2%, attack level 1.0-1.4. Highest damaged ratio in the hybrid combinations is only 20.5-56%, attack level 1.6-1.9.

In 2000 stem canker due to Cryophonectia at low level appeared in some hybrid combinations, mainly UE, EU and some E.exseta trees. In 2001 attack level remains as previously but the ratio of damaged trees is markedly lowered. The highest ratio of damaged trees found in two hybrid combinations E_1 U_{29} and E_4 U_{29}, is only 5-10% , next is C_{ken} : 10%. No attack is found in other hybrid combinations and other parent species.

5. Pulp potential of some eucalypt hybrids.

Research on pulp potential is carried out with some representative eucalypt hybrids at 3 years of age such as U_{29} E_1, E_1 U_{29}, U_{29} C_4 and E_1 C_4 together with their parent species (E1, U29, Ct) and sources used in production (E_1 . U_{29} . Ct . U_{Egon} C_{Ken}).

Table 1: Pulp potential of some eucalypt hybrids 3-years old at Ba Vi.

Hybrid combi-nations	Wood density	Stem volume (kg/tree)	Cellulose content (%)	Pulp effi-ciency after bleaching	Ratio (%)	Strength of paper after bleaching		
						Pulling strength	Doubled folding	Tearing index nNm^2/g
E_1C_4	0.511	6.42	45.5	41.9	73.8	6480	407	8.1
$U_{29}C_4$	0.495	12.21	47.3	43.1	80.4	6890	287	7.4
$U_{29}E_1$	0.499	15.84	48.1	46.2	79.3	5740	137	7.6
E_1U_{29}	0.496	11.69	48.3	42.1	80.9	4690.	274	6.6
U_{29}	0.437	3.62	47.2	42.3	75.2	5920	125	6.1
E_1	0.490	2.96	47.1	42.5	69.8	6310	156	6.6
Ct	0.472	8.46	45.4	43.7	75.5	5810	241	8.2

The studied eucalypt hybrid combinations all have wood specific density greater than the parent species (Table 1). On the other hand, due to fast growth, wood volume of hybrid combinations UE, EU and UC is also far greater than EC and the parent species. Hybrid combinations also have cellulose content, pulp efficiency higher than while lignin content and mechanical properties of paper are equal to the parent trees directly involved in hybridization.

6. Plus tree selection from eucalypt hybrid combinations.

Although most of hybrids do have growth faster than that of the parents directly involved in hybridization but there are only some hybrid combinations having marked faster growth than the best trees from seed source used in production planted as controls. Moreover of these combinations only some individual trees have best growth, finest stem form and good resistance to diseases.

Table 2: Average stem volume of some plus trees of some best eucalypt hybrid combinations selected at Ba Vi

Hybrid combinations	Selected at Ba vi			Comparison with trees from seed sources used in production (%)		
	D (cm)	H (m)	V (dm^3/tree)	D	H	V
Area planted 1998.						
Trees from seed source used in production (U_{SX}).	7,7	10,3	27,5	100	100	100
$U_{29}E_1$	10,8	13,3	63,7	140,3	129,1	231,6
$U_{29}E_2$	10,4	12,8	54,8	135,1	124,3	199,3
$U_{29}U_{26}$	10,2	13,2	54,2	132,5	128,1	197,1
$U_{29}C_4$	9,5	12,6	44,4	123,4	22,3	161,5
Area planted 1999						
Trees from seed source used in production (U_{SX}).	5,0	6,0	7,0	100	100	100
$U_{15}C_4$	8,4	8,8	24,8	168,0	146,7	354,3
GU	8,7	8,7	26,7	174,0	145,0	381,4
$U_{29}E_2$	7,9	9,0	22,1	158,0	150,0	315,7
$U_{30}E_5$	7,7	9,1	21,3	154,0	151,7	304,3

From the trials and trial planting in 2-3 years at Ba Vi there have been selected some individual trees in 12 hybrid combinations that have fastest growth, best tree stem quality and good resistance to diseases. Of these 12 hybrid combinations 4 are at 3 years of age and 4 are at 2 years of age. Average stem volume of some selected trees (Table 2) can be 1.6-3.8 arials that of trees from best seed source used in production and planted in the trial.

7. Propagation of eucalypt hybrids by meristem tissue culture.

Propagation of eucalypt hybrid $U_{29}C$ by meristem tissue culture using 2 upper most sections (1.0-1.5 cm long) from shoot of 3 month old ramets from cuttings. Research results show that sterilization with $HgCl_2$ 6.1% for the shoot section 2 in 8 minutes is most effective. Suitable shoot propagation medium for UC is MS (Murashige and Skooge) added with 0.5 mg/l BAP (Benzylaminopurine) and most effective rooting medium is MS alded with 1mg/l IBA (Acid Indol Batiric) (Doan Thi Mai et al 2000).

IV. Conclusions

From preliminary results of hybridization of some eucalypt species some conclusions can be made as follows:

1. E.urophylla flowering period does not coincide with that of E.camaldulensis and E.exserta. To obtain hybrids between E.urophylla with other two species, artificial hybridization must be carried out.

2. Seed of E.urophylla, E.camaldulensis and E.exserta can be stored at -30^c in 4 years.

3. Self-pollination in the 3 studied eucalypt species does not produce seed. Free pollination brings about high fruiting level but few seed are formed in the fruit. Controled pollination results in greatest number of seed in the fruit and weight of 1000 seed varies with the parents involved in hybridization.

4. The hybrid trees studied have morphological, anatomical indices and peroxydase intermediate between the two parents. Relative size of the branch is smaller than the parent trees directly involved in the

hybridization.

5. In the first 2-3 years almost all eucalypt hybrid combinations in the trials have growth faster than that of the parent trees directly involved in the hybridization. Of the hybrid combinations, some have marked faster growth than the parents and trees from seed source used in production.
6. Expression of heterosis in growth varies according to the planting conditions. Where the soil is good eucalypts grow fast and heterosis is markedly expressed. Where the soil is poor, eucalypts grow slowly and heterosis manifests itself poor.

7. Many hybrid combinations have stronger resistance to diseases and pest insects than the parent trees directly involved in the hybridization. Bark canker in eucalypt manifests itself at low level and the resistance to diseases of hybrids is not higher than that of the parents.

8. At 3 years of age the eucalypt hybrid combinations all have specific density of wood and cellulose content higher than the parent trees directly involved in hybridization. Some combinations have higher pulp efficiency than the parents while paper strength is equal to that of the parents.

9. From the trials some eucalypt hybrids have been selected with productivity 60-200% higher than trees from seed source used in production, fine stem form and free from disease and insect attack.

10. Propagation of eucalypt hybrid by meristem tissue culture is effective when H_gCl_2 is used for sterilization of shoot section 2 in 8 minutes. Shoot propagation medium is MS added with 0.1mg/l BAP. Rooting medium is MS added with 1mg/l IBA.

References

1. Doan Thi Mai, Nguyen Viet Cuong, Ngo Thi Minh Duyen, Nguyen Thien Huong, 2000. Preliminary results of eucalypt hybrid propagation by meristem tissue culture. Forestry Review No 10 p. 46-48.

2. Le Dinh Kha, 1970. A new form of eucalypt of fast growth in North Vietnam. Forestry Review, No 3. p. 27-34.

3. Le Dinh Kha, 1996. Research on creating a scientific and technological foundation for the supply of improved forest planting stock. Final scientific report of research subject KN 03.03. Forest Science Institute of Vietnam, 63 pp.

4. Le Dinh Kha, et al. 2001. Breeding and propagation of some main forest tree species. Final scientific report of research subject KHCN 08.04. Forest Science Institute of Vietnam. 174 pp.
5. Moran. G.F. and Bell. J.C. 1983. Eucalyptus Isozyme in plant genetics and breeding. Tansley S.P. and Orton T.J. Ed. Amsterdam - Oxford - New York. p. 423-442.

6. Martin. B., 1989. The benifits of hybridization. How do breed for them. Breeding Tropical Trees. Population structure and genetic improvement strategies in clonal and seedling forestry. Workshop in Pattaya. Thailand. p.72-92.

7. Nguyen Duong Tai, 1994. Preliminary trial of Eucalyptus urophylla provenances in paper raw material area of Mid-region of North Vietnam. Thesis for Doctor of Agricultural Science.

8. Pham Van Tuan, Nguyen Hoang Nghia, Le Dinh Kha, Hoang Chuong, 2000. Results of eucalypt species and provenances trials in Vietnam. Paper submitted to conference for Recognition of eucalypt and acacia planting stock 17. p.p.

9. Paramathma, M. and Surendran, C.2000. Utilisation of hybrid vigour in Eucalyptus. Hybrid Breeding and Genetics of Forest Trees. QFRI/CRC - SPF. Symposium Noosa. Queensland. Australia 9-14 April. p. 467-471.

10. Sharma Jyoti, K.1994. Pathological investigations in forest nurseries and plantations in Vietnam. UNDP/FAO. VIE/92/022. Hanoi 46 p.p.

11. William, E.R., and A.C. Matheson. 1994. Experimental Design and Analysis for use in Tree Improvement. CSIRO. Melbourne and ACIAR. 174. p.p.

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