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Variations in Peroxidase and Nitrate Reductase Activities and Growth of *Populus alba* × *Populus glandulosa* F_1 Clones[1]

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第一代 雜種포플러, *Populus alba* × *Populus glandulosa* F_1 클론의 過酸化酵素, 窒酸還元酵素의 活性變異 및 生長에 關한 硏究[1]

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ABSTRACT

To slect the superior clones of *Populus alba* × *Populus glandulosa* F_1, growth and some growth-related enzyme activities were examined for thirteen, two-year-old and fifteen, three-year-old trees at Seoul National University nursery in Suwon. Clonal differences in total dry weight per tree and leaf surface area per tree were significant at the 5% level. Significant correlations were found between total dry weight per tree and leaf surface area per tree (r=0.875), between leaf peroxidase activity per tree and total dry weight per tree (r=0.854), and between leaf nitrate reductase activity per tree and total dry weight per tree (r=0.914). Leaf peroxidase and nitrate reductase activities per unit fresh weight of one-year-old tree increased with increasing leaf order numbers basipetally, reaching maximum values in the eighteenth and thirty-third leaves, respectively, and decreased gradually from those leaves to basipetal lower leaves. Clones 65-29-19, 66-15-3, 65-22-11, 66-14-93, and 66-26-55 among two-year-old trees, and clones 64-6-44, 66-14-29, 66-26-55, 65-22-11, and 68-1-54 among three-year-old trees showed greater leaf surface areas, peroxidase and nitrate reductase activities per unit leaf fresh weight than other clones, Growth of *Populus alba* × *Populus glandulosa* F_1 clones might be estimated from either leaf surface area per tree or peroxidase and nitrate reductase activities per tree. Therefore, measurements of leaf surface area and leaf enzyme activities appear useful to select superior *Populus* clones at early growth stages.

Key words : Populus alba × P. glandulosa; *peroxidase; nitrate reductase; clone; activity.*

要 約

第一代 雜種 포플러 *Populus alba* × *P. glandulosa* F_1의 優良 clone을 選拔하기 위해, 서울大學校 農科大學 苗圃場에서 密植栽培(20,000本/ha)한 2, 3年生 各各 13, 15clones을 對象으로 生長과 이에 關聯된 過酸

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化酵素 및 窒酸還元酵素의 活性을 調査·比較하였다. 個體當 乾重量·個體當 葉面積에 있어서는 clone間 有意差가 認定되었으나, 葉單位生重量當의 過酸化酵素 및 窒酸還元酵素의 活性値는 有意差가 認定되지 않았다. 個體當 乾重量과 個體當 葉面積과의 相關關係는 r=0.875, 個體當 乾重量과 個體當 葉의 過酸化酵素의 活性値와는 r=0.854, 個體當 乾重量과 個體當 窒酸還元酵素의 活性値와는 r=0.914로 高度의 相關을 보였다. 1年生 clone의 葉部位別 葉單位生重量當의 過酸化酵素와 窒酸還元酵素의 活性値는 葉順序가 위에서부터 아래로 내려옴에 따라 增加하여 18번째와 33번째에서 各各 最大値를 보였으며, 그 후 아래로 내려올수록 減少하는 傾向을 보였다. 2年生 clones 65-29-19, 66-15-3, 65-22-11, 66-14-93, 66-26-55 등과 3年生 clones 64-6-44, 66-14-99, 66-26-55, 65-22-11, 68-1-54 등이 個體當 葉面積値, 葉單位面積當 過酸化酵素 및 窒酸還元酵素의 活性値가 다른 clone들보다 높아서 良好한 生長能力을 가졌다고 말할 수 있다. 第一代 雜種 포플러, *Populus alba*×*P. glandulosa* F_1의 幼時生長을 個體當 葉面積, 個體當 葉의 過酸化酵素 및 窒酸還元酵素의 活性値로 推定可能性을 보였으며, 이러한 事實로 보아, 葉面積과 葉의 過酸化酵素 및 窒酸還元酵素의 測定値는 本 雜種 포플러의 優良 clone을 選拔하는 하나의 基準으로 利用될 可能性을 보인다.

INTRODUCTION

Plant growth is the result of dry matter produced in all of life processes controlled by genes and environment. However, genes are mediated by enzymes. Thus, the enzymes regulating certain life processes contribute greatly to determining ultimate yield. Certain enzyme activities may be logical criteria for selecting superior species or clones. Peroxidase (PER) and nitrate reductase (NR), as key enzymes, have shown promise of being useful for growth rate prediction.[5,6,7,21,22]

Peroxidase, a hemoprotein that catalyzes the oxidation of substrates by peroxide, can be extracted from plant cells and organs, and has shown on active metabolic role. Most of the peroxidases are relatively unspecific to hydrogen donors, which include phenolic substances, cytochrome c, ascorbate, indolacetic acid, amines and certain inorganic ions, and in some respects, mimic the oxidases specific for these substrates. However, they have an absolute requirement for peroxide as oxidant. Their metabolic roles vary with the tissue in which they are found. Their potential roles in development, besides that of lignification,[11] are indicated by peroxidase activity in the oxidation of indoleacetic acid,[16,18,21] ethylene biosynthesis and disease resistance. Peroxidase activity in herbaceous and woody plants is affected by growth hormones[16,22] and seems to be closely related to growth, differentiation, lignification,[11,18] and aging.[16,19,22] Therefore it seemed sensible to investigate the use of peroxidase activity and isozyme patterns in selecting woody species and clones for rapid growth. Early selection for inherently rapid growth rate might be enhanced by including peroxidase information in the selecting system.

Most common and abundant source of nitrogen for plants is the nitrate ion. Nitrate is absorbed and reduced first to nitrite, and then to ammonium ions. The ammonium ions are subsequently converted into glutamic acid and then to hundreds of other essential organic nitrogen compounds. First step in conversion of nitrate to ammonium involves the enzyme, nitrate reductase.

Nitrate reductase is substrate inducible,[2,13] and the reduction of nitrate to ammonium is the rate-limiting step. Higher levels of enzyme would enhance the input of reduced nitrogen for protein synthesis in plants.[1,4,7] Dykstra[6] suggested that a better understanding of relationships between nitrogen uptake and biochemical factors may provide useful indices for rapid screening and selection of genotypes more required to substrate nitrogen. Ingle[13] also reported that they could use nitrate reductase activity as a measure of nitrogen requirement of trees. Eilrich and Hageman[7] found that with field grown Arthur wheat the input of reduced nitrogen estimated from nitrate reductase activity of the entire canopy was significantly related to the amount of reduced nitrogen of the

total shoot tissues. Deckard *et al.*[5] also suggest that the precision of the nitrate reductase assay as a selection tool is also improved by the use of related genotypes.

The objective of this study was to find some enzymatic activities related to fast growth and high yield, which can be used for selecting superior *Populus alba* × *P. glandulosa* F_1 clones at early stages. The specifie objectives were to investigate 1) clonal and seasonal variation in peroxidase activity of the *Populus* leaf and subtending internode, 2) clonal variation in nitrate reductase activity of the *Populus* leaf.

MATERIALS AND METHODS

1. Plant Materials

Two-and three-year-old *Populus alba* × *P. glandulosa* F_1 clones were provided as plant materials. Two- and three-year-old *Populus* plantations and experimental designs were the same as those used in the previous study (Kim and Lee, 1983).[15]

2. Analysis of Total Peroxidase Activity

Peroxidase analysis was performed with 9th to 12th leaves in basipetal direction and its subtending internode on lateral shoots. Leaf and internode were excised and 0.5 g of tissue sample taken from each part was ground with 5 ml of extraction buffer (0.05 M Tris-HCl, pH 7.4, 0.1 M sucrose). The resulting homogenate was centrifuged (0°C, 1 hr, 30,000 × g).

Total peroxidase activity was determined by measuring the rate of decomposition of hydrogen perioxide with 3,3′-dimethoxybenzidine, as the hydrogen donor and dye. The reaction mixture for the total peroxidase activity included 1 ml of 0.3% H_2O_2 plus 99 ml of buffered 3,3′-dimethoxybenzidine solution prepared by diluting 0.8 ml of 1% 3,3′-dimethoxybenzidine in methanol to be 100 ml with 0.01 M phosphate buffer (pH 6.0). After 0.1 ml of supernatant was mixed with 2.9 ml of reaction mixture in a quartz cuvette, the rate of color development at 460 mm was determined in one minute interval during 3 minutes with Spectrophotometer (Shimadzu 200-200). Total peroxidase activity was expressed increasing absorbance per minute, as Gregory did.[9] Monthly measurements was performed triplicated per clone from mid-June to mid-September. Clonal mean values shown in this paper was calculated from triplicated measurements.

3. Analysis of Nitrate Reductase Activity (*in Vivo* Assay)

Nitrate Reductase (NR) activity was measured by modified Jaworski's method.[14] Fresh leaf tisses (0.2 g) were rinsed with cold tap water followed by cold deionized water and suspended in a screw cap vial(25 ml) containing 5 ml of medium solution consisting of 0.1 M phosphate buffer (pH 7.5), 0.02 M KNO_3, 5% propanol and two drops of chloramphenicol (0.5 mg/ml). The vials were sealed and incubated in the dark at 28°C for 2 hours. Nitrite released into the medium was determined by treating 0.4 ml aliquots with 0.3 ml of 1% sulfanilamide in 3 M HCl and 0.3 ml of 0.02% N-1-naphthyl-ethylene diamine hydrochloride solution. After 20 minutes the solutions was diluted with 4 ml of water and the optical densities was measured at 540 nm. Nitrate reductase activity was expressed as μ mole of nitrite formed per gram of fresh tissue per hour. Monthly measurements was performed triplicated per clone on late-August and late-September. Clonal mean values shown in this paper was calculated from triplicated measurements.

4. Leaf Surface Area and Total Dry Weight

Every third leaves were systematically sampled to measure leaf length and width in mid-August and mid-September for two-year-old clones. Leaf surface areas were estimated by the method described by Kim and Lee[15]. To measure total dry weight for two-year-old clones, six cuttings per clone were harvested in late-October. Total dry weights of all tree components including roots were measured after drying at 80°C in an oven over 72 hrs.

RESULTS AND DISCUSSION

1. Enzyme Activities of the three-year-old *Populus* clones

Table 1 shows total peroxidase and nitrate reductase activities per 0.5 g fresh tissue of the three-year-old clones. Both of the enzyme activities were not significantly different at the 5% level between clones, but seasonal differences in activities were significant at 1% level.

The highest value for total peroxidase activity of the leaf tissue was 0.804 (ΔO.D.460 nm/min.) in September and the lowest, 0.387 in July. In clonal comparison, clone 66-14-99 showed the highest value, 0.679, and clone 66-6-8 did the lowest, 0.480. Overall mean value for 15 clones was 0.557.

The highest value for total peroxidase activity of the subtending internode was 0.369 in August, and the lowest, 0.327 in June. For clonal comparison, clone 65-95 showed the highest value, 0.420 and clone 67-6-3 did the lowest, 0.284.

Table 1. Mean values of enzyme activities in three-year-old *Populus alba* × *P. glandulosa* F_1 clones

Clones	Peroxidase activity (Δ O.D. 460nm/min.)										Nitrate reductase activity in leaves (NO_2^- μ mole-g^{-1} fresh wt. $hr.^{-1}$		
	Leaf					Subtending internode							
	Jun.	Jul.	Aug.	Sep.	Mean	Jun.	Jul.	Aug.	Sep.	Mean	Aug.	Sep.	Mean
64-6-44	.411	.396	.611	.943	.588	.411	.423	.306	.398	.385	6.21	9.33	7.77
65-22-4	.441	.407	.643	.883	.594	.410	.305	.281	.345	.335	6.17	6.71	6.44
65-22-11	.286	.546	.641	.918	.598	.343	.312	.345	.368	.342	5.99	7.42	6.71
65-29-19	.425	.406	.434	.960	.556	.453	.331	.310	.395	.372	7.11	6.48	6.80
65-95	.313	.367	.527	.749	.500	.317	.613	.427	.323	.420	6.27	6.11	6.19
66-6-8	.293	.372	.664	.590	.480	.479	.383	.429	.315	.402	6.01	8.80	7.41
66-14-29	.382	.348	.737	.803	.566	.375	.350	.413	.313	.365	6.65	8.23	7.44
66-14-93	.456	.410	.513	.658	.509	.441	.388	.311	.305	.361	6.93	6.41	6.67
66-14-99	.709	.374	.830	.804	.679	.164	.346	.625	.389	.381	6.55	9.16	7.86
66-15-3	.439	.410	.601	.691	.535	.246	.326	.420	.378	.368	6.23	9.04	7.64
66-20-1	.469	.358	.866	.810	.626	.420	.259	.434	.317	.358	5.93	6.16	6.27
66-25-5	.211	.376	.543	.841	.493	.246	.289	.299	.322	.289	5.87	7.79	6.83
66-26-55	.331	.441	.776	.833	.570	.251	.355	.408	.353	.342	6.51	9.09	7.80
67-6-3	.368	.338	.647	.898	.563	.146	.340	.263	.390	.284	5.88	6.83	6.36
68-1-54	.391	.260	.693	.673	.504	.209	.371	.267	.348	.299	6.90	7.10	7.00
Mean	.395	.387	.648	.804	.557	.327	.359	.369	.351	.354	6.35	7.64	7.01

Overall mean value of the subtending internode is much smaller than that of the leaf tissue.

For nitrate reductase activity clone 66-14-99 as in the case of peroxidase showed the highest value, 7.85 (NO_2- μ mole. g^{-1} fresh wt. hr^{-1}) and clone 65-95 did the lowest value, 6.19.

2. Total Dry Weight, Leaf Surface Area, and Enzyme Activities of the two-year-old *Populus* clones.

Table 2. shows total peroxidase activity, nitrate reductase activity, total dry weight, and leaf surface area of two-year-old *Populus* clones. Clonal differences in total dry weight were significant at 5% level, and clone 65-29-19 was the highest whereas clone 66-14-29 was the lowest. Clonal differences in leaf surface area per tree were also significant, and clone 65-29-19 was the highest and clone 66-14-29 the lowest. These results were similar to those reported by Kim and Lee (1983)[15].

Clonal means of the enzyme activities of the two-year-old clones were not significantly different at the 5% level, but their seasonal means were different at 1% level. Total peroxidase activity in the leaf tissue of clone 66-14-99 was the highest among all the clones and that of clone 66-6-8 was the lowest.

Table 2. Mean values of total dry weight leaf area and enzyme activites in two-year-old *Populus alba* x *P. glandulosa* F_1 clones

Clones	Total dry weight (g)	Leaf area (cm^2)			Peroxidase activity (Δ O.D. 460 nm/min.)										Nitrate reductase activity (NO_2^- μ mole · g^{-1} fresh wt. $hr.^{-1}$)		
					Leaf					Subtending internode							
		Aug.	Sep.	Mean	Jun.	Jul.	Aug.	Sep.	Mean	Jun.	Jul.	Aug.	Sep.	Mean	Aug.	Sep.	Mean
64-6-44	556	11030	6213	8622	.360	.297	.398	.568	.406	.211	.316	.452	.235	.304	6.23	6.83	6.53
65-22-4	445	11791	5335	8563	.272	.321	.414	.598	.401	.287	.243	.268	.533	.333	6.13	6.13	6.13
65-22-11	824	16077	12876	14476	.254	.458	.260	.693	.416	.246	.368	.240	.213	.292	5.91	5.99	5.95
65-29-19	891	17206	13879	15542	.390	.524	.420	.705	.408	.258	.279	.263	.233	.258	6.85	8.16	7.51
65-95	590	9004	5736	7370	.325	.499	.411	.515	.435	.300	.564	.327	.293	.371	6.58	7.10	6.84
66-6-8	540	14126	8700	11414	.287	.367	.302	.350	.327	.272	.341	.286	.308	.327	5.85	6.12	5.93
66-14-29	154	5623	2979	4302	.285	.379	.334	.608	.337	.346	.360	.317	.258	.320	6.39	5.58	5.99
66-14-93	571	12774	7731	10253	.257	.331	.268	.640	.399	.200	.366	.240	.253	.275	5.93	5.92	5.93
66-14-99	490	9456	7156	8598	.326	.420	.367	.718	.458	.212	.373	.302	.383	.293	6.92	7.25	7.09
66-15-3	539	10304	8316	9310	.284	.287	.267	.625	.391	.379	.262	.308	.315	.316	6.38	8.03	7.21
66-20-1	402	10550	6036	8292	.251	.478	.237	.570	.382	.247	.317	.253	.290	.250	5.96	8.53	7.16
66-25-5	575	7793	6329	7061	.266	.437	.358	.753	.429	.298	.523	.291	.250	.341	6.16	5.58	5.87
66-26-55	564	11040	10565	10802	.281	.414	.334	.660	.420	.256	.345	.308	.228	.309	5.79	5.73	5.76
Mean	549	11290	7836	9585	.295	.401	.344	.615	.389	.270	.358	.297	.291	.307	6.24	6.67	6.44

For seasonal comparison, mean value was the highest in September, while that was the lowest in June.

Total peroxidase activity in the subtending internode of clone 65-95 was the highest among all the clones and that of clone 66-20-1 was the lowest. For seasonal comparison, mean value in July was the highest, while that in June was the lowest.

Nitrate reductase activity of the leaf tissue of clone 65-29-19 as in the case of total dry weight and leaf area showed the highest value, 7.51, wherease clone 66-26-55 did the lowest, 5.76.

Peroxidase activity in the leaf and subtending internodes was reported to vary with various position and aging of the tissue extracted,[19,22] extraction and assay method,[3,17] and physiological status of the tissue.[18] Peroxidase activities were nearly similar when assayed repeatedly on the same extract, but varied greatly among the extracts and among the clones, probably indicating that there are too small to detect the clonal differences in peroxidase activity, and that there exist the difficulty of attaining accurate fresh weight and accurate extraction with mortar and pestle. Similar trend in poplar was also shown by Wray.[22]

Nitrate reductase activity of the leaf tissue in this study was varied greatly and was not significantly different among clones. This results are different from the clonal differences and genetically controlled nitrate reductase in hybrid poplar reported by Fasehun.[8] It seems that genetically controlled differences in nitrate reductase activity too small to detect or to show clonal differences, and that any fertilization was not applied in this field study. Nitrate reductase activity of unit fresh leaf could not used as a selection tool, judging from the results. However, considerably more detailed work would have to be done. This suggestion is also supported by earlier observations in hybrid poplar,[6,8] and crop species.[4,5,7]

3. Variations in Fresh and Dry Weight, Total Peroxidase and Nitrate Reductase Activities of the Leaf for one-year-old Plants

Table 3 and Figure 1 showed mean values of total peroxidase and nitrate reductase activities in leaves, and leaf fresh and dry weight per unit area according to the basipetal leaf order for one-year-old clones 65-22-11, 65-29-19, and 66-20-1. To compare with the variation of chlorophyll content and photosynthetic ability in relation to basipetal leaf order reported previous,[15] these data was

Table 3. Mean values of leaf weights, peroxidase activity and nitrate reductase activity by basipetal lead number in one-year-old *Populus alba* × *P. glandulosa* F_1 clones

Basipetal leaf order number	Leaf Weight ($g \cdot cm^{-2}$)		PER. Activity (Δ O.D. 460 nm/min)	NR. Activity (NO_2^- μ mole g^{-1} fresh wt. hr^{-1})
	Dry	Fresh		
3/47	4.61	13.74	.401	5.47
8/47	4.98	13.30	.385	5.54
13/47	4.59	11.10	.402	5.56
18/47	4.31	10.85	.449	5.68
23/47	4.02	11.05	.421	5.86
28/47	3.85	10.50	.409	6.23
33/47	3.72	10.54	.416	7.59
38/47	3.95	11.90	.378	7.42
43/47	4.90	13.82	.354	6.68
Mean	4.33	11.87	.402	6.23

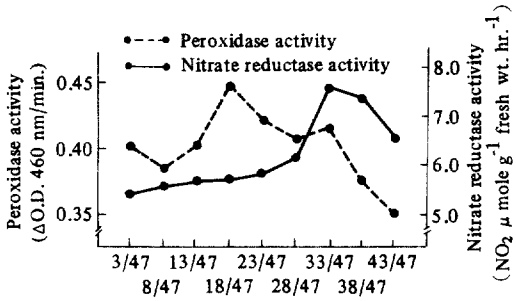

Fig. 1. Peroxidase and nitrate reductase activities measured in mid-August in relation to basipetal leaf order in one-year-old *Populus alba* × *P. glandulosa* F_1 clones.

measured in mid-August. Total peroxidase and nitrate reductase activities increased with increasing leaf order up to the eighteenth and thirty-third, respectively, and then decreased. It suggests that thirty-third leaves retain highest protein synthetic capacity. Such results were similar to those reported by Harper and Hageman, [12] but disimilar to those reported by Fasehun,[8] the younger leaves had higher nitrate reductase activity than old leaves. This discrepancy was probably derived from the differences in growing stages and experimental conditions, such as field and greenhouse, soil type, photoperiod, fertilization, etc. Canopy profile of nitrate reductase activity was similar to that of chlorophyll content reported Kim and Lee.[15]

4. Relationships among Tatal Dry Weight(G), Leaf Surface Area(LA), Peroxidase Activity of the Leaf(PL), Peroxidase Activity of the Subtending Internode(PS), and Nitrate Reductase Activity of the Leaf (NR).

Regression equations among total dry weight(G), leaf surface area(LA), peroxidase activity of the leaf(PL), peroxidase activity of the subtending internode(PS), and nitrate reductase activity of the leaf(NR) were shown in Table 4. Peroxidase activity of the leaf per tree(PLT) and nitrate reductase activity of the leaf per tree(NPT) were calculated from the values of PL and NR multiplied by leaf surface area.

Low correlations were found between G and PL, between G and PS, and between G and NR. However, high correlations were found between G and LA (r=0.875), between G and PLT (r-0.914), and between G and NRT (r=0.854). Scatter diagrams shown in Figure 2,3,4, also indicates the close relationship between G and LA, between G and PLT, and between G and NRT, respectively. Low correlations between G and PS, and G and NR differed from the report by Wray[22] and Fashun,[8] respectively. High correlation of G and NRT was similar to those observed in crop species.[4,5,7]

From these results, peroxidase and nitrate reductase activities of unit fresh tissues (PL, PS and NR) were inadequate for selection tool for fast growth characters. However, leaf surface area(LA), Peroxidase activity of the leaf per tree(PLT), and

Table 4. The relations among means of total dry weight (G), leaf area (LA), peroxidase activity of the leaf(PL), peroxidase activity of the subtending internode(PS), nitrate reductase activity of the leaf (NR), peroxidase activity of the leaf per tree (PLT), and nitrate reductase activity of the leaf per tree (NRT) in two-year-old *Populus alba* × *P. glandulosa* F_1 clones

Predicted Equation	R^2	F-value	t-values for β's	
			β_1	β_0
G = .05232 LA + 47.851	.7656	36.062**	6.0052**	.549
G = 1514.9 PL − 62.352	.0729	.861	.9279	−.591
G = −1240.2 PS + 929.87	.0543	.631	.7946	1.931
G = 58.547 NR + 171.46	.0467	.476	.6898	.364
G = .13219 PLT + 35.451	.8350	55.677**	7.4617**	.659
G = .00708 NRT + 109.42	.7292	29.612**	5.4417**	1.306

* and ** indicate 5% and 1% significance levels, respectively.

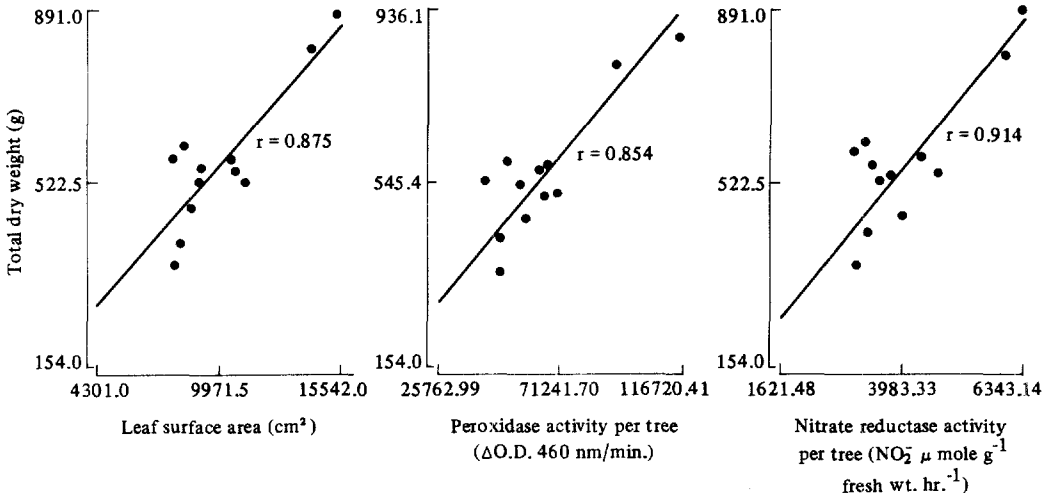

Fig. 2. Relationship between total dry weight per tree and leaf surface area per tree.

Fig. 3. Relationship between total dry weight per tree and peroxidase activity per tree.

Fig. 3. Relationship between total dry weight per tree and nitrate reductase activity per tree.

nitrate reductase activity of the leaf per tree(NRT) could be useful criteria for identifying fast growth characters.

CONCLUSION

Two of the growth-related enzyme activities and other growth characters were examined during early growing period to help select superior *Populus alba* × *P. glandulosa* F_1 clones. Clonal differences in leaf surface area and total dry weight per tree were highly significant at the 1% level in two-year-old clones. However, differences in total peroxidase activity of the leaf and subtending internode tissue per unit fresh weight, and nitrate reductase activity of the leaf per unit fresh weight were not significant between clones at the 5% level.

Total dry weight per tree was strongly correlated with leaf surface area per tree, peroxidase activity per tree, and nitrate reductase activity per tree.

When all of the clones are ranked, based on leaf surface area, peroxidase activity and nitrate reductase activity per unit leaf fresh weight, they can be grouped in the following Table 5.

The following equations can be used to estimate total dry weight yield of *Populus* clones

Table 5. Clonal grouping in growth potential, based on leaf area, peroxidase activity, and nitrate reductase activity per unit leaf fresh weight

Two-year-old plants			
Leaf area	PER. activity	NR. activity	Clones
High	High	High	65-29-19, 65-15-3
High	High	Low	65-22-11, 66-14-93, 66-26-55
High	Low	High	
High	Low	Low	66-6-8
Low	High	High	64-6-44, 66-14-99, 65-95
Low	High	Low	65-22-4, 66-25-5
Low	Low	High	66-20-1
Low	Low	Low	66-14-29

Three-year-old plants		
PER. activity	NR. activity	Clones
High	High	64-6-44, 66-14-29, 66-14-99, 66-26-55
High	Low	65-22-4, 65-22-11, 66-20-1, 67-6-3
Low	High	66-6-8, 66-16-3, 68-1-54
Low	Low	65-29-19, 65-95, 66-14-93, 66-25-5

G = .05232 LA + 47.851 (r=0.875)

G = .13219 PLT + 35.451 (r=0.854)

G = .00708 NRT + 109.42 (r=0.914)

, where G. LA, PLT, and NRT indicate total dry weight per tree, leaf surface area per tree in square centimeter, total peroxidase activity of the leaf per tree, and nitrate reductase activity per tree, respectively.

From above results, clones 65-29-19, 66-15-3, 65-22-11, 66-14-93, and 66-26-55 for two-year-old plots have better growth potential than the others.

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