

Cone and seed characteristics in the second generation seed orchard of *Pinus thunbergii*

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Introduction

What is seed orchard?

- A stand created by selecting trees with excellent traits distributed nationwide
- Stable supply of genetically modified superior seeds
- The bridge of tree breeding result, planting and forestry management



Selective tree breeding phase

Research background

Seed orchard

- Improving productivity and quality of Important of improved seed producti seed from Seed orchard
- Fertility, conelet & seed production contribution analysis of constituent species
- Grasp of cone and seed production

Seed

- The seed from seed orchard supply r Self-sufficiency rate of timber ate is about 30 percent \rightarrow study on ways to increase
- Necessity of establishment a seedling foster and forestation of commercial productions system.
- on and supply

Productivity improvement

- Korea's forest area \rightarrow 64.1% of total la nd area
- \rightarrow 16.1% (2016' statical yearbook of f orestry)
- forest for stable supply and demand of wood



Self-sufficiency rate Wood supply and demand

Research subject species

- Pinus thunbergii (Korean black pine , 海松)
 - A representative tree native to the coast of Korea
 - Status of plus tree selection : 151
 - First generation Seed orchard Area : 22 ha
 - Second generation Seed orchard Area : 3 ha
- Korean black pine progeny test
 - open pollinated progeny test : 69 family, 1.48 ha
 - controlled-pollinated progeny test : 317 combination, 3.8 ha
 - → As a result of controlled-pollinated progeny test between the composition clone of first generation seed orchard, create second generations seed orchard by selecting combinations with excellent growth

Ratio of Pine and Korean Black pine forest to stocking land (%)



• Korean black pine

- ✓ Physiological and morphological similarities with pine trees.
- ✓ For the next three years, Fifth of the reforestation plan among conifer trees (249 ha)

Preceding research

- In Korea, there was a study of characteristic of clone, species and seed productions of first generations seed orchard.
- Since the second generations seed orchard in 2006, a lack of research on changes in genetic diversity associated with flowering variations, characteristic of cone or seed.
- Hereafter, it will be necessary to study about advanced generation seed orchard, such as pine trees, nut pine, oak trees which are major commercial species.

Research purpose

- 1) From seed orchard creations to seed production, proper management plan is important(location selection, arrangement of clone, roguing, etc)
- 2) Through cone analysis, improving seed quality by analyzing the genetic characteristics of the seeds between matching combinations
- 3) As the first 'second generations seed orchard' of Korea, provide the basic data necessary for the establishment and management(e.g. roguing, artificial pollen supply)



Testing materials : Korean Black pine second-generation seed orchard

- Creation site : 51-1, Jungjangri, Anmyeon-eup, Taean-gun, Chungcheongnam-do
- Creation year : 2006
- Creation area : 1 ha
- mating combinations : 26 combinations (075 x 0636 etc)
- planting interval : 5m x 5m (square planting)



The frond view of black pine second generation seed orchard

Data collections : Cone collections and morphological characteristic research

- Date and time : September 2014, 2015, 2016 (total 3 times)
- Collection : Total 390 cones(26 combination x 5 cones x 3 times)
- Research traits : lively cone length(mm), width(mm), weight(g) measurement



Korean black pine cone collection and dry

Investigation item of Korean black pine cone characteristics

Cone analysis : Fertile scale elimination and seed separation



Fertile scale elimination and seed separation



aborted ovule

(Lee et al, 1984)

aborted ovule

Analysis item : the number of scale, seed per cone

- scale = fertile scale + non-fertile scale
- seeds = developed ovule + aborted ovule
- seed potential, total developed seeds, % of filled seed, % of aborted ovules, seed efficiency and % of germination (Calculated as follows)
 - \checkmark Seed potential = fertile scale \times 2
 - \checkmark Total developed seeds = filled seeds + empty seeds
 - \checkmark Percent of filled seeds = filled seeds / total developed seeds \times 100
 - \checkmark Percent of aborted ovules = aborted ovules / seed potential \times 100
 - \checkmark Seed efficiency = total filled seeds / seed potential \times 100
 - ✓ Percent of germination = $\frac{Germinated seeds}{Filled seeds} \times 100$

(USDA Forest service, 1977; Lee et al. 1984)

Statistical analysis : IBM SPSS Statistics 23

1) One-way ANOVA

• Calculate variance component(σ_e^2 , σ_c^2) and heritability(h^2) based on MS

Source of variation	df	EMS	h^2
Among clones	C – 1	$\sigma_e^2 + k\sigma_c^2$	σ_e^2
Within clones	N – C	σ_e^2	$\overline{\sigma_e^2 + \sigma_c^2}$

C = Number of clones, N = Number of seed orchard tree

(Kang et al., 2001)







Comparison of cone (2015)

Morphological characteristics of cone by year or combination

	Length				Width		Weight			
	2014	2015	2016	2014	2015	2016	2014	2015	2016	
Mean	55.4	51.5	60.0	31.4	29.7	31.7	18.1	22	28.4	
S.D.	5.32	5.7	6.37	3.31	2.46	2.77	5.13	5.73	6.95	
Min	43.3	41.1	52.8	23.7	23.5	23.61	8.6	11.6	16.1	
Мах	70	73.3	77.8	40.4	38	39.4	31.3	48.9	53.8	
CV	0.083	0.098	0.088	0.097	0.072	0.079	0.251	0.224	0.210	
Ns	25.8	25.7	25.8	25.8	25.9	25.8	24.5	24.7	24.9	
Nr(%)	99.3	99.0	99.3	99.0	99.5	99.4	93.9	95.2	95.8	
GD	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	

✓ 2016 : Cone length, width, weight were excellent

 $\checkmark\,$ 2015 : Most of cones length, width were the lowest

2							
	1	2	3	4	5	6	7
ω	0214*0661	0224*063	0233*0643	0614*0229	0619*062	0619*083	0621*0817
4					6600		
	8	9	10	11	12	13	14
7	0634*077	0634*081	0638*076	0638*083	0640*094	0641*073	0644*0646
8 9 10							
	15	16	17	18	19	20	21
ゴ目	0644*087	0648*0815	072*045	072*0636	075*045	075*0636	075*0641
12 13 14							0001
	22	23	24	25	26		↔
5	076*0641	076*092	087*0645	096*0638	096*083		1cm
16 17 18				0311	0000		

Filled-seed, empty-seed, aborted-ovule forms comparison by combinations (2015)

Analysis of cones by year

	Seed	Aborted	Percent	Number	of develo	Percent	Seed		
year	potential	ovule	aborted ovule(%)	total	Empty seeds	Filled seeds	filled seeds(%)	efficiency (%)	
2014	80.4	20.0	24.9	60.4	13.1	47.3	77.6	58.8	
2015	80.7	15.8	18.9	65.0	10.0	55.0	84.2	68.5	
2016	96.5	25.5	23.9	71.0	6.8	64.2	90.1	68.6	

Estimation of variance component and heritability(h²) per year by cone analysis

	2014				2015				2016			
	F	σ_c^2	$\sigma_e{}^2$	h²	F	σ_c^2	$\sigma_e{}^2$	h²	F	σ_c^2	$\sigma_e^{\ 2}$	h²
Length	10.58***	19.1	10.2	0.651	12.84***	15.2	28.8	0.345	7.09***	201.3	2,292.9	0.081
Width	19.43***	8.5	2.7	0.759	13.37***	7.2	34.7	0.171	10.65***	8.7	18.8	0.316
Weight	12.34***	20.9	7.0	0.748	12.39***	31.2	37.2	0.456	10.60***	37.9	30.0	0.558
Seed potential	7.08***	125.8	207.0	0.378	4.42***	68.8	141.1	0.328	17.48***	293.2	262.1	0.528
% developed seeds	11.63***	157.5	67.8	0.699	14.13***	113.0	55.0	0.672	10.52***	89.4	125.0	0.417
% filled seeds	11.49***	159.8	182.8	0.466	7.50***	69.3	118.9	0.368	4.16***	53.0	162.5	0.246
% aborted ovules	16.96***	148.5	50.5	0.746	14.13***	154.8	145.5	0.515	10.63***	179.8	245.8	0.422
Seed efficiency	10.63***	180.3	134.1	0.573	13.57***	139.4	71.4	0.661	9.82***	182.7	157.1	0.538

* : p<0.05, ** : p<0.01

✓ Cone and seed characteristics show the statistical difference between the combinations.

✓ Cone and seed characteristics : under strong genetic control (high heritability)



Result summary

- ✤ Morphological characteristic of cone :
 - 2014 : average cone length 55.4mm, width 31.4mm, weight 18g
 - 2015 : average cone length 51.5mm, width 29.7mm, weight 22g
 - 2016 : average cone length 60.0mm, width 31.7mm, weight 28.4g
- ✤ Analysis of cone and seed characteristic :
 - 2014 : seed potential 80.4, aborted ovule 24.9%, filled seeds 77.6%, seed efficiency 58%
 - 2015 : seed potential 80.7, aborted ovule 18.9, filled seeds 84.2%, seed efficiency 68.5%
 - 2016 : seed potential 96.5, aborted ovule 23.9%, filled seeds 90.1%, seed efficiency 68.6%

✤ Estimation of variance analysis and heritability(h²) :

- Statistical significance is recognized between combinations.
- Cone and seed characteristics were under strong genetic control.
 Especially, seed efficiency is more than 0.5 per year
 - → under very strong genetic control

Conclusions

- 1. Cone analysis and seed characteristics analysis result
 - \checkmark There is a difference in seed quality between year, combinations
 - \rightarrow Selective cone collection
 - ✓ Excellent heritability to cone and seed characteristics
 - \rightarrow Selection index for advanced generation breeding
- Practical application to the reasonable and efficient management of seed orchard
- Need to grading(normal, first or second generation) of seeds from seed orchard and price differential research



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Thank you