The effect of half-sib lines on morphological attributes of one-year old *Fraxinus angustifolia* seedlings

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Abstract

Aiming to produce forest reproductive material at the family level, we tested quality of twenty *Fraxinus angustifolia* Vahl half-sib lines in the nursery experiment. We measured height (HT) and root collar diameter (DIA) of one-year old seedlings, and we monitored germination rate and mortality during the first growing season. Seeds of 20 half-sib lines originating from Sombor area (north Serbia) were sown in seedbed on autumn 2016. The seed dormancy was not broken in all seeds equally, resulting with un-uniform germination over a two months period. All measured attributes shows a statistically significant differences, indicating a strong effect of genetic control. The highest average and DIA were measured in half-sib line 7 (22.62 cm; 5.79 mm, respectively). The lowest average DIA was measured in half-sib lines 18 (2.85 mm) and 19 (2.94 mm), which can be result of growing density effect (104 seedlings m^{-2} for line 18 and 126 seedlings m^{-2} for line 19, compared to 40 seedlings m^{-2} for line 7). The correlation between DIA and HT is strong and positive (R=0.90). Due to its morphological superiority, half-sib line 7 could be recommended for mass production of *Fraxinus angustifolia* seedlings at the family level.

Keywords

Half-sib line; Seedbed density; Seedlings Quality; Narrow leaved ash

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1 Introduction

Fraxinus angustifolia Vahl is a wind-pollinated tree, an important component of European mixed broadleaved woodlands. It is closely related to *F. excelsior*, to the extent that they are able to hybridize. According to Fraxigen (2005) narrow-leaved ash occurs naturally throughout most of the Iberian peninsula; on the Mediterranean coasts of France, Italy and the Balkan peninsula as far as northern Greece; south into coastal areas of Morocco, Algeria and Tunisia; and east as far as the Caspian Sea.

Given the assumptions about the global increase of the temperature areal of the Narrow-leaved ash could be extended (Fraxigen 2005).

In Serbia, two regions of provenance were designated for narrow-leaved ash species based on distribution, plant communities and climate characteristics (Cvjetićanin et al. 2014). Provenance region One consists of narrow-leaved ash forests (*Fraxinus angustifolia* Vahl) in Podunavlje and Posavina in Vojvodina and in Posavina, Pomoravlje and Podunavlje in Central Serbia at the altitudes up to 100 m (Cvjetićanin et al. 2014). Species is widely distributed in monodominant and mixed forests in the alluvial area of large rivers (Tomić 2004) found in coastal and floodplains (Jovanović 2007) and covers an area of 25.200 ha (Banković et al. 2009). *F. angustifolia* is a pioneer species on gley soils, where build monodominant forests (Bobinac et al. 2010). Also, it is an endangered species and there is not enough information about *F. angustifolia* stands and variability in Serbia (Čortan et. al. 2017).

Resistant, high quality and good health condition seedlings are the primary goal of planting material production. The present emphasis is on adaptive rather than productive traits, through a strong focus on local sources of planting material. Trees planted in the context of ecological restoration, or enrichment of native woodlands, require different qualities for adaptation and competition from those established in plantations specifically for timber production (Fraxigen 2005). However, the selection of source for production of planting material should not be limited to local provenances, but rather extended to the best families according to survival and growth rates.

Knowledge of genetic variability, especially adaptive traits (growth, survival, and phenology) is the basis for the production of planting material (Booy et al. 2000; Schaberg et al. 2008; Šijačić-Nikolić and Milovanović 2010; Isajev and Šijačić-Nikolić 2011; Isajev et al. 2011; Ivankovć et al. 2011; Šijačić-Nikolić et al. 2012; Balian et al. 2015; Ivetić et al. 2016; Ivetić and Devetaković 2017). Due to lack of natural regeneration, production and planting of narrowed-leaf ash seedlings is crucial for regeneration of stands on the floodplains (Andrić 2018).

Grading and quality testing of seedlings at operational level are mostly based on morphology (Mohammed 1997) and physiology (Mattsson 1997), with seedlings height and root collar diameter as the most used attributes (Stilinović 1960). Measurement of both height and diameter is nondestructive, fast, and easy and most often used to assess the quality of seedlings (Ivetić 2013).

The objective of this study was to select the best half-sib lines for production of *Fraxinus angustifolia* seedlings based on survival and growth rates.

2 Material and methods

Seedlings are produced from seed collected in Zapadno-Bački District - Sombor area (north Serbia), from 20 seed trees in a single stand, resulting with 20 half-sib lines. Seeds are collected in October2016 and sown in seedbed with natural soil. The seeds from each half-sib line were sown in four repetitions along the seedbed to eliminate the effect of different microsite conditions. During the growing season, cultural practices of a manual weeding and hoeing were performed once a week. The seedbeds were irrigated when needed, based on soil moisture.

The total number of seedlings (NS) was counted from July to November once per month, because un-uniform germination. At the end of growing season we measured growing density (number of seedlings per m^2) for each half-sib line, height (HT) and root collar diameter (DIA) of all seedlings in the seedbed (total sample of 944 seedlings), resulting with an unequal sample size. The HT was measured as distance between the root collar and the base of terminal bud of dormant seedlings, with an accuracy of 0.1 cm. DIA was measured at or near the root collar, with an accuracy of 0.1 mm (Ivetić 2013).

Descriptive statistics (mean value, standard deviation (SD), variation coefficient (CV%) and minimum and maximum values), analysis of variance (ANOVA) and LSD test (Fisher's least significant difference test) of measured morphological attributes were statistically analyzed using „STATGRAPHICS Centurion XVI". Relation between DIA and HT was examined by correlation coefficient (R) for bivariate data with scatter diagrams showing linear association using Statistica 7 software (StatSoft, Inc. 2004).

3 Results

Measured morphological attributes showed large variability (Table 1). The results showed that differences between studied attributes were statistically significant between 20 selected half sib lines ($p < 0.05$).

Table 1. Summary statistics and ANOVA of *Fraxinus angustifolia* one-year seedlings morphological attributes: NS – number of seedlings, MV – mean value, standard deviation in parenthesis, CV – coefficient of variation, min-max - minimum and maximum values of measured attributes, mean values followed by a different letter are significantly different (LSD test).

SUMMARY STATISTICS							
		Diameter [mm]			**Height [mm]**		
Half-sib line	*NS*	*MV* [mm]	*CV* [%]	*Min-max* [mm]	*MV* [mm]	*CV* [%]	*Min-max* [mm]
1	9	4.15 (0.60)^{CDEFG}	14.50	2.92-4.98	143.89(43.23)^{CDEFG}	30.04	92-240
2	44	4.79 (1.35)^{GH}	28.12	2.42-7.87	177.82(61.24)^{GH}	34.44	90-357
3	42	5.50 (2.06)^{I}	37.43	1.82-11.42	224.98(89.32)^{IJ}	39.70	100-560
4	60	5.74 (2.38)^{I}	41.40	2.12-12.51	247.52(116.86)^{J}	47.21	96-537
5	68	4.82 (1.87)^{GH}	38.84	1.39-10.77	200.03(81.36)^{H}	40.67	63-410
6	32	5.52 (1.67)^{I}	30.27	2.85-9.96	198.34(81.70)^{HI}	41.19	91-428
7	40	5.79 (2.25)^{I}	38.77	1.78-12.06	226.20(84.04)^{J}	37.15	52-374
8	25	3.85 (1.20)^{DEF}	31.12	1.32-6.58	152.76(57.09)^{FG}	37.37	89-320
9	36	3.76 (1.69)^{DE}	44.90	0.66-7.21	124.92(39.13)^{BCDEF}	31.33	53-210
10	30	5.28 (1.28)^{HI}	24.22	2.16-8.46	145.40(46.69)^{EF}	32.11	58-263
11	34	4.58 (1.43)^{FGH}	31.17	2.39-9.43	133.29(49.31)^{DEF}	36.99	60-285
12	45	3.61 (1.10)^{CDE}	30.61	1.96-7.48	110.33(39.99)^{ABCD}	36.25	63-267
13	64	3.12 (0.83)^{ABC}	26.62	1.19-4.83	113.95(38.11)^{ABCD}	33.44	37-224
14	34	3.60 (1.16)^{CDE}	32.33	1.01-6.95	116.35(42.10)^{ABCDE}	36.18	50-221
15	9	3.52 (1.27)^{ABCDEF}	36.10	1.92-6.31	79.56(27.13)^{A}	34.10	45-118
16	57	3.20 (0.94)^{ABCD}	29.57	1.48-5.68	95.30(24.98)^{A}	26.21	47-181
17	44	3.41 (1.25)^{BCD}	36.76	1.35-6.6	100.75(34.74)^{AB}	34.48	35-229
18	104	2.85 (1.09)^{A}	38.32	0.74-6.28	99.056(37.09)^{A}	37.44	25-236
19	126	2.94 (1.17)^{AB}	39.73	0.85-6.38	104.01(37.90)^{ABC}	36.44	40-252
20	41	4.15 (1.52)^{EF}	36.62	1.17-8.53	129.93(53.80)^{DEF}	41.41	68-306
Total	944	4.02 (1.79)	44.60	0.66-12.51	143.17(77.30)	53.99	25-560
		Between half-sib lines for diameter (mm)			**Between half-sib lines for height (mm)**		
ANOVA statistics		*Mean square*	*F-ratio*	*P-value*	*Mean square*	*F-ratio*	*P-value*
		53.21	24.44	0.0000	124979.0	35.42	0.0000

The average coefficient of variation per half sib line for DIA was about 33% (in total 44.6%), and for HT was about 36% (In total 53.99%). The highest average HT and DIA were measured in half-sib line 7 (226.2 cm; 5.79 mm, respectively). The lowest average DIA was measured in half-sib lines 18 (2.85 mm) and 19 (2.94 mm),which can be result of growing density effect (104 seedlings m^{-2} for line 18 and 126 seedlings m^{-2} for line 19, compared to 40 seedlings m^{-2} for line 7) (Table 2).

Table 2. Growing density (number of seedlings per m^2) for each half-sib line for all counting times.

Counting date	Half sib line																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
	Number of seedlings per m^2																			
04.07.2017	46	65	44	58	73	37	38	38	38	35	35	61	73	35	13	64	46	82	68	50
04.08.2017	44	64	44	58	71	34	38	28	34	32	32	54	69	34	13	57	42	112	110	46
04.09.2017	21	45	44	61	70	33	38	26	34	32	32	54	67	39	10	55	42	112	113	42
04.10.2017	11	43	37	54	65	30	36	22	32	31	29	48	56	29	6	50	37	93	98	41
05.11.2017	9	44	42	60	68	32	40	25	36	30	34	45	64	34	9	57	44	104	126	40

Relation between DIA and HT for *Fraxinus angustifolia* one-year seedlings is strong and positive (R=0.90) (Figure 1), NS and DIA is moderate and negative (R= -0.35) (Figure 2, left), and between NS and HT is weak and negative (R=-0.11) (Figure 2, right).

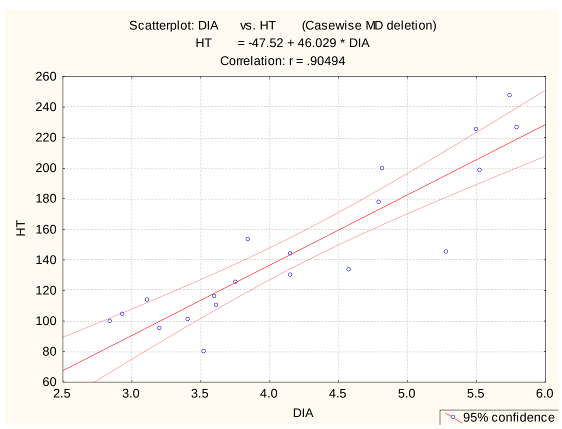

Figure 1. Relationship between HT:DIA.

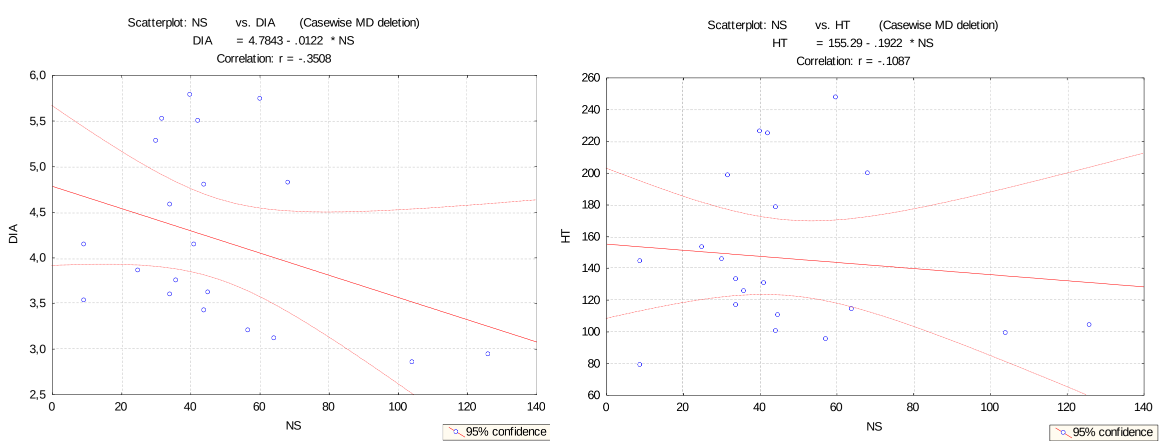

Figure 2. Relationship between NS:DIA (left) and NS:HT (right).

4 Discussion

According to Fraxigen (2005), identifying superior reproductive material for production and genetic improvement programmes for narrow-leaved ash remain important at both national and regional levels. First step of genetic improvement process starts with the evaluation of seed sources (Fraxigen 2005), like we did in this research by selecting 20 half-sib lines. The number of half-sib lines in reforestation determines the degree of genetic diversity and adaptability and depends on the seed source (seed stands or orchards), seed processing and nursery production (Ivetić et al. 2016). In many studies was found that genetic variability for Narrow-leaf ash populations is high (Jarni 2009; Jarni et al. 2011; Papi et al. 2012; Jeandroz et al. 1996; Kremer et al. 2010; Čortan et. al. 2017; etc.), which means that narrow-leaf ash have a great genetic potential for conservation and directed utilization. An inherent high genetic variability determine great heterogeneity of seeds behavior and germination (Yücedağ and Gailing 2012), in which is supported by results of our research.

It is known that lower seedbed increase root collar diameter, with or without reducing height, increase dry weights, decrease shoot to root ratio, and improve field performance (as reviewed by Ivetić and Devetaković 2016). Yücedağ and Gailing (2012) in their research found the negative effect of seedbed density on growth of *F. angustifolia*. They explained this by the increasing competition among the seedlings for light, water, and nutrition. The similar results are reported in a number of studies (Cengiz and Şahin 2002; Çiçek et al. 2007; Semerci et al. 2008), as well as in our research, with negative correlation between NS and growth of one-year of *F. angustifolia* seedlings.

Monitoring the seedlings quality is important in forest nurseries, because better understanding and application of cultural practices that improve seedling quality and promote the reforestation success (Ivetić et al. 2017). According to Ivetić et al. (2016B) both initial HT and DIA should be considered equally important in operational programs for hardwoods seedling quality testing, because initial height and diameter are equally related to survival and growth of hardwood seedlings in first year after field planting.

Obtained values for HT and DIA are in range of previous research of narrow-leaved ash seedlings (Bobinac et al. 2010; Ivetić et al. 2014; Drvodelić et al. 2016; Çiçek et al. 2007; Çiçek et al. 2010). However, results of this research recommend half-sib line 7 for mass production of *Fraxinus angustifolia* seedlings at the family level due to its morphological superiority.

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