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Evergreen azaleas tolerant to neutral and basic soils: breeding potential of wild genetic resources

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Abstract

Evergreen azaleas (*Rhododendron* subg. *Tsutsusi*) are ornamental plants which usually grow in acidic soils. They perform best when the pH ranges between 4.5 and 6.0. On calcareous soils, they often show strong iron-deficiency chlorosis symptoms and their growth is inhibited. Therefore their cultivation area is limited. However, wild azaleas were seen to show different adaptability to soil pH, according to species and habitat environment. With a long-term goal of selecting commercial genotypes tolerant to pH higher than optimum, we collected plant materials in the wild and in historical gardens and we performed specific crosses. To evaluate their neutral-basic pH tolerance, seeds were placed to germinate at nine different pH regimes ranging from 2 to 8.5. Ultra acid pH inhibited seed germination especially in *R. macrosepalum, R. ripense* and *R. japonicum*. However a clear correlation between germination capacity and pH was not observed. Genotype dependent differences were better observed by a screening, in which rooted cuttings were grown in nutritive solutions at three pH regimes (6.0, 7.5, and 9.0). *R. indicum* showed serious chlorosis symptoms in all conditions. By contrast, *R. macrosepalum* var. *hanaguruma* and *R. scabrum* were tolerant to neutral-basic pH, showing very limited leaf damage. As confirmed by molecular analyses, these two latter species belong to the subsection *Macrosepala* and are close related to *R. ripense*, whose elevated pH tolerance was already known. These wild genetic resources appear promising for pH tolerance breeding efforts.

INTRODUCTION

Evergreen azaleas (*Rhododendron* subg. *Tsutsusi*) are important ornamental plants which usually prefer acid soils. They perform best when the pH is between 4.5 and 6.0. Often they show strong iron-deficiency chlorosis symptoms on calcareous soil (Giel and Bojarczuk, 2002). The presence of calcium carbonate ($CaCO_3$) in the substrate, via alkalinization of the rhizosphere, markedly hinders their growth (Giel and Bojarczuk, 2011). Elevated HCO_3^- concentrations and low Fe availability are therefore among the most serious factors limiting azalea cultivation and use in landscape. However, genetic variability for pH tolerance exists even within species. Wild azaleas showed different adaptability to soil pH, according to the species and the environment from which they came from. In particular, *R. ripense* is known to be tolerant to alkali substrates (Scariot and Kobayashi, 2008; Kobayashi and Scariot, 2008).

With a long-term goal of breeding commercial genotypes tolerant to pH higher than optimum, we collected plant materials in the wild and in historical gardens. Specific crosses were made and seed germination as well as rooted cuttings response to different pH regimes were evaluated.

MATERIALS AND METHODS

Seed germination

A total of 324 open pollinated seeds *per* species were collected from *R. japonicum*, *R. kiusianum*, *R. kaempferi*, *R. macrosepalum*, and *R. ripense*, treated with 50 ppm GA_3 for 1 day, and then sowed on polyurethane sponges filled with NaOH or H_2SO_4 solutions to obtain the following pH regimes: 2, 2.5, 3, 3.5, 5, 7, 7.5, 8, and 8.5. In order to maintain the pH values stable during the experiment, solutions were renewed once a week. A constant temperature of 25°C and a daily photoperiod of 16h (53 µmol $m^{-2}s^{-1}$) with cool, white fluorescent lamp were applied.

The number of germinating seeds was recorded weekly for 7 weeks. Seeds were considered germinated when a visible radical protrusion reached approximately 2 mm (Bhatt and Ram, 2007). The final germination percentage (FGP) and the time of 50% germination (*T50*) were calculated and the means were post hoc tested using Ryan-Einot-Gabriel-Welsch-F test (REGW-F), by means of the SPSS statistical package (version 17.0; SPSS Inc., Chicago, USA).

Hydroponic screening

Ten azaleas were selected for this trial: *R.* x *purchrum* 'Oomurasaki', *R. scabrum, R. macrosepalum* 'Hanaguruma', *R.* x *mucronatum* 'Ryukyushibori', *R.* x *mucronatum* 'Fujimanyo', *R. indicum*, *R. indicum* 'Juko', *R. indicum* 'Shinsen', *R. obtusum* 'Susogo no ito', and *R. tosaense*.. Ten rooted cuttings *per* genotype were placed in three different solutions: the first (standard solution) contained deionized water and 0.5 g/L of Peter's fertilizer (20:20:20) with pH 6; in the second and third solutions, 0.10 g/L and 1.00 g/L of anhydrous $NaHCO_3$ were added at the standard solution to adjust the pH to 7.5 and 9.0. After three weeks, chlorophyll content (SPAD units), number of leaves open at the base, percentage of leaf damage (0=0%, 1≤5%, 2=6-25%, 3=26-75% and 4=≥76%), height, root size, and plant mortality were evaluated. Data were subjected to the analysis of variance (ANOVA) using Ryan–Einot–Gabriel–Welsch's multiple step-down F (REGW-F) test, ($P \leq 0.05$). The Kruskal-Wallis test was used to investigate differences in visual damages and root quality. All statistical analyses were performed using SPSS statistical package (version 17.0; SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Seed germination

In natural habitats, the soil pH from the root zone of *R. kiusianum*, growing in active volcanic area, can be very low (3.9). The soil pH of *R. kaempferi* and *R. macrosepalum* populations, growing on the edges of secondary forest and hillside, generally ranges between 4.2 and 5.7 while, some populations of *R. ripense* located along stony river banks grow at pH 7.6 (Scariot and Kobayashi 2008). However, in the present study, the germination test showed few differences related to pH tolerance among species, with seeds from all species germinating in all pH condition above 3.0 (Table 1). Overall, FGP mean values were highest in *R. japonicum*, *R. macrosepalum*, and *R. kaempferi* (45.3%, 43.8% and 43.5%, respectively) while lowest in *R. ripense* (30.8%) and *R. kiusianum* (20.4%).

The time to reach 50% germination was generally 2 weeks regardless of pH, except for *R. kiusianum* at pH 7.0 and *R. kaempferi* at pH 5.0 were it was 3 weeks. For *R. japonicum,* T_{50} was always reached after 1 week.

Hydroponic screening

The screening of rooted cuttings in hydroponic conditions indicated that significant genotypic variations exist in relation to pH (Table 2).

R. scabrum resulted pH tolerant, growing well in all conditions with very low leaf damages (always below 1.5) and mortality rate. Similarly, *R. macrosepalum* ‘Hanaguruma’ (damage class = 2.8 at pH 9.0) and *R. indicum* ‘Juko’ (damage class = 2.8 at pH 7.5) showed restrained leaf damages at higher pH. In contrast, *R. indicum* ‘Shinsen’ showed serious symptoms and high mortality even at acidic pH (pH 6.0). This sensitivity to hydroponic solutions at pH 6.0 was also noted in *R.* x *mucronatum* ‘Fujimanyo’, *R. tosaense*, and *R. indicum*, which showed symptoms with damage values superior than 2.3 and a mortality rate higher than 50 % of treated cuttings, showing leaf abscission already after one week (data not shown).

In the other genotypes, differences among the three pH regimes were mainly observed after three weeks (Table 2). *R.* x *purchrum* ‘Oomurasaki’ and *R.* x *mucronatum* ‘Ryukyushibori’ showed a rather limited leaf damage (less than 25% at pH 9) but a reduction in leaf number already at pH 7.5, as well as in *R. obtusum* ‘Susogo no ito’.

Plant height was mainly stunted in *R. obtusum* ‘Susogo no ito’ at pH 7.5 and 9.0. A reduction in root development was induced in *R.* x *purchrum* ‘Oomurasaki’and *R.* x *mucronatum* ‘Ryukyushibori’ by pH 7.5 and 9.0 solutions, and in *R. obtusum* ‘Susogo no ito’ by pH 9.0. Lastly, plant mortality was generally high in *R.* x *mucronatum* ‘Ryukyushibori’ (50% at pH 9) and almost total in *R. obtusum* ‘Susogo no ito’ (70% at pH 7.5 and 90% at pH 9.0).

Overall, this screening allowed for the selection of three promising azalea genotypes putatively tolerant to neutral-basic pH conditions: *R. scabrum*, *R. macrosepalum* ‘Hanaguruma’, and *R. indicum* ‘Juko’. Intermediate pH susceptibility was shown by *R.* x *purchrum* ‘Oomurasaki’and *R.* x *mucronatum* ‘Ryukyushibori’. In contrast, *R.* x *mucronatum* ‘Fujimanyo’, *R. obtusum* ‘Susogo no ito’, *R. tosaense*, *R. indicum*, and *R. indicum* ‘Shinsen’ proved to be the most susceptible showing chlorosis, leaf abscission, and root browning in the elevated pH treatments. However these results need to be validated in soil conditions.

CONCLUSIONS

R. scabrum and *R. macrosepalum* ‘Hanaguruma’ proved to be the most tolerant to neutral-basic pH root zone conditions. These two species are taxonomically grouped in the subsection *Macrosepala* together with *R. ripense*, a species already demonstrated to be tolerant to elevated pH conditions. *R.* x *purchrum* ‘Oomurasaki’and *R.* x *mucronatum* ‘Ryukyushibori’ have also *R. ripense* in their pedigrees and may be eligible for further investigations. Variability was observed within *R. indicum* species. To better understand the molecular bases of stress tolerance, other screening and analyses by means of molecular markers are in progress.

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Tables

Table 1. Effects of pH on the germination capacity of five azalea species. Values refer to the FGP (final germination percentage) and T_{50} (time of 50% germination).

Species	**pH**	**FGP (%)**	T_{50} **(days)**	**Species**	**pH**	**FGP (%)**	T_{50} **(days)**
R. japonicum	2.0	05.5 b[§]	7	*R. macrosepalum*	2.0	05.6 d	7
	2.5	11.1 b	7		2.5	13.9 cd	14
	3.0	41.6 a	7		3.0	44.4 ab	14
	3.5	41.6 a	7		3.5	38. 9 bc	14
	5.0	55.5 a	7		5.0	66.7 a	14
	7.0	58.3 a	7		7.0	50.0 ab	14
	7.5	66.7 a	7		7.5	61.1 ab	14
	8.0	63.9 a	7		8.0	50.0 ab	14
	8.5	63.9 a	7		8.5	63.9 ab	14
P		*		*P*		*	
R. kiusianum	2.0	00.0 b	-	*R. kaempferi*	2.0	00.0 c	-
	2.5	16.7 ab	7		2.5	25.0 bc	14
	3.0	27.8 ab	14		3.0	44.4 ab	14
	3.5	13.9 ab	14		3.5	52.8 ab	14
	5.0	16.7 ab	14		5.0	63.9 a	21
	7.0	19.4 ab	21		7.0	41.7 ab	14
	7.5	25.0 ab	14		7.5	50.0 ab	14
	8.0	27.8 ab	14		8.0	66.7 a	14
	8.5	36.1 a	14		8.5	47.2 ab	14
P		*		*P*		*	
R. ripense	2.0	05.6 cd	7				
	2.5	02.8 d	7				
	3.0	13.9 bcd	7				
	3.5	41.7 ab	14				
	5.0	47.2 a	14				
	7.0	47.2 a	14				
	7.5	33.3 abc	14				
	8.0	44.4 a	14				
	8.5	41.7 ab	14				
P		*					

[§] Mean values showing the same letter are not statistically different at $P \leq 0.05$ according to the REGW-F test.

Table 2. Main effects of pH on SPAD values, number of leaves, leaf damages (classes), plant height, root length, and mortality as observed after 3 weeks in different azalea (Cv).

Cv	pH	SPAD values	Leaves (n.)	Leaf damages	Height (cm)	Root length (cm)	Mortality (%)
R. indicum	6.0	-	1.6	3.8	9.2a	2.6a	80
	7.5	-	0.5	3.8	8.6a	2.2a	90
	9.0	-	1.8	3.6	3.3b	0.6b	70
	P		ns	ns	**	*	ns
R. indicum 'Juko'	6.0	53.34	6.8	0.7	9.9	3.5	0
	7.5	57.64	3.4	2.8	9.1	3.8	10
	9.0	55.55	5.0	2.0	9.3	2.7	30
	P	ns	ns	ns	ns	ns	ns
R. indicum 'Shinsen'	6.0	45.05	0.1	3.7	9.4	3.1	90
	7.5	48.88	1.2	3.1	9.7	3.4	70
	9.0	41.31	0.3	3.7	9.1	3.0	90
	P	ns	ns	ns	ns	ns	ns
R. macrosepalum 'Hanaguruma'	6.0	45.31	4.1	1.5	9.2	3.5	20
	7.5	44.54	3.5	1.4	10.0	2.3	0
	9.0	48.42	2.9	2.8	10.4	3.2	30
	P	ns	ns	*	ns	ns	ns
R. obtusum 'Susogo no ito'	6.0	-	4.3a	1.4	11.1a	3.3a	10
	7.5	-	1.3b	3.2	9.3b	3.7a	70
	9.0	-	0.2b	3.6	8.4b	1.8b	90
	P		**	**	**	**	**
R. scabrum	6.0	54.78	4.8	1.1	10.4	1.9	0
	7.5	49.31	3.7	1.5	9.0	1.6	10
	9.0	49.27	3.2	0.9	8.5	0.7	0
	P	ns	ns	ns	ns	ns	ns
R. tosaense	6.0	-	1.8a	2.6	9.4	1.6	60
	7.5	-	1.6a	2.7	9.6	1.9	60
	9.0	-	0.0b	4.0	9.4	1.4	100
	P		*	*	ns	ns	*
R. x *mucronatum* 'Fujimanyo'	6.0	-	3.3a	2.3	10.2	3.3	50
	7.5	-	2.0ab	2.9	10.7	3.1	70
	9.0	-	0.0b	4.0	10.6	3.6	100
	P		*	*	ns	ns	*
R. x *mucronatum* 'Ryukyushibori'	6.0	47.26	5.0a	1.3	11.1	4.4a	10
	7.5	47.36	2.9b	1.7	10.8	2.0b	10
	9.0	43.44	1.5b	2.5	10.7	2.7b	50
	P	ns	*	*	ns	*	*
R. x *purchrum* 'Oomurasaki'	6.0	50.15	7.5a§	1.2	11.4a	4.2a	10
	7.5	46.20	2.4b	2.6	10.0b	3.3b	40
	9.0	46.67	4.5b	2.0	10.9ab	3.1b	20
	P	ns	*	ns	*	*	ns

§ Mean values showing the same letter are not statistically different at $P \leq 0.05$ according to the REGW-F test.