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Mutation breeding using gamma-rays to increase seed germination in *Rosa hybrida*

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Abstract

Breeders are always looking for new and novel varieties to be competitive in the flower market. Low seed germination rate is still a major problem encountered in traditional rose breeding programs. Mutagenic agents, such as ionizing radiation, may be used to enhance seed vigor and increase the productivity, improving sprouting and emergence of buds that are carried out through seed coating. The effects of ionizing radiation on seed vigor are in most cases genotype-dependent, thus irradiation treatments need to be optimised for each different cultivar. In the present study, gamma-rays (0, 50, 100 and 200 Gy) were applied on hybrid tea rose seeds in order to set up a radiation protocol for increasing seed germination. Seeds coming from six different crossings of *Rosa hybrida* commercial cultivars were used in order to gain information on radiotolerance and germination ability.

INTRODUCTION

Hybrid tea roses (*Rosa hybrida*) are the most important ornamental cut-flowers worldwide. Breeders are always looking for new and novel varieties to be competitive in the flower market. Rose breeding programs center mainly on the introduction of new flower colors, thornless stems, higher production, and good post-harvest performance (Pipino 2011). Rose propagation by seed is used in breeding new cultivars as well as in the production of rootstock plants. Poor seed germination is one of the major problems encountered in rose breeding programs (Anderson and Byrne 2007). This is mainly due to endogenous and exogenous dormancy (Pipino et al. 2012, Bosco et al. 2014), which requires prolonged and expensive treatments (Zlesak 2006, Pipino et al., 2011) or techniques (Caser et al., 2012, Caser et al. 2014) to be overcome.

Mutation breeding is the process of exposing seeds to chemicals or radiation in order to generate mutants with desirable traits to be bred with other cultivars. Mutagenic agents are commonly used to generate genetic variation and thus select the desired characters with a global impact of mutation-derived varieties on food production and quality enhancement (Ahloowalia et al., 2004). Recent reports have underlined the potential of gamma rays as tools for seed priming, a process used in seed industry to increase seed vigor and to enhance plant tolerance to biotic/abiotic stresses (Macovei et al., 2014). Exposing plants to radiation is sometimes called radiation breeding, the biological effects of ionizing radiation (IR) are correlated with the energy absorbed per unit mass (dose) and, for a given dose, to the rate of energy deposition (dose rate) (Vilenchik and Knudson, 2000). The optimal dose is based on radiosensitivity, which is dependent both on genotype and physiological conditions of plant material (Esnault et al., 2010). Gamma-rays have been successfully exploited to enhance seed vigor in okra and hard wheat (Hegazi and Hamideldin, 2010; Melki and Marouani, 2010), however the irradiation dose should be carefully assessed to avoid the occurrence of undesirable effects. In *Rosa hybrida*, few studies on mutagenesis with ionizing radiation have been carried out. Ibrahim et al. (1998) applied X-irradiation at 25, 50 and 100 Gy on leaf explants for adventitious bud regeneration. Authors observed a decreasing rate of leaf explants regenerating buds with increasing dose (from 47% to 0%). Recently, *in vitro* radio-sensitivity of leaf explants of *R. hybrida* was tested with different doses of gamma-irradiation (0-50 Gy) (Kahrizi et al., 2013). Authors found that the LD_{50} for leaf explants is 20-30 Gy. No studies on seed mutagenesis are currently present in literature.

In the present work, gamma-rays (0, 50, 100 and 200 Gy) were applied to hybrid tea rose seeds in order to investigate the potential use of ionizing radiation as a tool to improve seed quality and increase seed germination.

MATERIALS AND METHODS

Seed collection and irradiation

Six crosses of *R. hybrida* were made by manual pollinations in the greenhouses of NIRP International (New International Rosebushes Protected, Bevera, Ventimiglia, IM, Italy) during June 2012 (Table 1). A total of 200 seeds were extracted from mature hips of each cross and dry seeds were separated in four groups (50 seeds each) and exposed to 50, 100, and 200 Gy doses of gamma rays from Co^{60} gamma source (high dose rate = 5.15 Gy min^{-1}) available at the University of Pavia. Untreated controls (NT, fifty seeds) were used.

Seed germination

Irradiated and control seeds were sowed in perlite seed beds for germination in the greenhouses of the NIRP International in February 2013. On the basis of the International Rules for Seed Testing Association (ISTA 2014), seed germination was weekly recorded for a total of four months. The final germination percentage (FGP), half-time of germination (T_{50}), and germination period (GPD) were calculated using Czabator’s index (1962) where FGP = total number of seeds germinated/total number of seeds in all replicates × 100; T_{50} = days from seeding to the 50% of the total germinated seeds, and GPD = days from seeding to when maximum number of seeds germinated. Moreover, the day of the first emergence (Em) and the germination energy (Ge) were counted. Germination energy is defined as the germination percentages when the mean daily germination (cumulative germination percent divided by the time elapsed since sowing date) reached its peak. Seeds were considered to have germinated when hypocotyl with cotyledons emerged.

Seed quality

For each tested irradiation dose, changes in final germination percentage (±%) were estimated in *Rosa hybrida* crosses in comparison with the untreated sample (NT). After 16 weeks from sowing, the following growth criteria were recorded: flowering time and the percentage of flowering seedlings on total germinated seeds (Fl).

Statistical analysis

Arcsine transformation was performed on all percent incidence data before statistical analysis in order to improve homogeneity of variance. Effect of radiation treatments on FGP, Em, T_{50}, GPD, and Ge were evaluated by the analysis of variance (one-way ANOVA) using Ryan-Einot-Gabriel-Welsch's multiple stepdown F (REGW-F) test ($p \leq 0.05$). All analyses were performed with SPSS 21.0 Inc. software (Chicago, Illinois, USA).

RESULTS

In Table 2 are reported the effects of radiation treatments on seed germination of the studied crosses. The final germination percentage (FGP) was not significantly affected by the treatments. Few differences were noted for the T_{50} and the GPD while the day of the first emergence (Em) varied in all the progenies, even if with different radiation-dose effect. The 50 Gy-dose shortened Em in the progenies of four out of six crosses: 8, 187, 325 and 331. The seed progeny coming from the cross 187 (3940 orange code x 1225 orange code) showed a positive trend in germination percentage at 50, 100 and 200 Gy, while seed germination of cross 331 (2466 red code x 3741 red code) was negatively affected by high dose radiation (Table 3). All the obtained seedlings were then monitored at flowering time. The percentage of seedlings which started flowernig in June 2013 is listed in Table 2. Although no significant differences were counted also for this parameter, seedlings obtained from seeds treated with 50 Gy showed a flowering percentage trend superior than the others in crosses 8 (Fl = 68%), 325 (Fl = 67%) and 331 (Fl = 75%). Furthermore in the cross 51, the 100% of seedlings obtained from 50 and 100 Gy treated seeds produced flowers.

DISCUSSION

High ionizing radiation doses (> 10 kGy) are routinely used for the sterilization of food products, while low doses, ranging from 60 to 700 Gy, are used to improve characters and productivity in many seed propagated crops, such as rice, wheat, maize, beans and rape seed (Ahloowalia and Maluszynski, 2001). In the present study we evaluated the effects of gamma-rays at 50, 100 and 200 Gy on seed quality and germination, in order to assess the response of six crosses of *Rosa hybrida* and acquire useful information for developing novel seed vigorization protocols.

The range of gamma rays doses tested did not significantly affect final germination percentage and germination energy of the seeds regardless to the cross. These results are in contrast with different studies performed on seeds of several species such as *Vigna unguiculata* L. (Bind and Dwivedi, 2014), *Citrus jambhiri* Lush. (Sharma et al., 2013) and *Withania somnifera* L. (Bhosale and More, 2014) where a gradual decrease in seed germination and seedling vigor from lower doses to higher doses in given treatments of gamma rays were detected. This is a controversial issue since other reports are available which demonstrate the beneficial effects of ionizing radiation on seed vigor. Few differences among rose radiated seeds were observed for the other evaluated parameters (T_{50}, GPD and Em). In particular, the 50 Gy dose fasten the emergency period. Only one cross showed a positive trend in the germination percentage related to the radiation dose applied, in agreement with Bottino et al. (1975) who highligted that relatively low-doses ionizing irradiation on plant materials accelerated seed germination rate. With the aim to better explain the potential of gamma ray-based biostimulation in hybrid tea rose, the range of total dose will be expanded and the effects of different dose rate conditions (low dose rate versus high dose rate) will be also conducted. Seeds from a wider number of *R. hybrida* crosses will be tested in order to acquire more detailed information on a large sample population. This will allow a better understanding of the cultivar-related ability to withstand genotoxic-stress as well as the seed profile in terms of stress tolerance and vigor.

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Tables

Table 1. Identification code (ID), mother plant and pollen donor of the six crosses of *Rosa hybrida*.

Cross ID	**Mother plant**	**Pollen donor**
8	2364 yellow code	3523 yellow code
51	3951 red code	4100 red code
72	3418 yellow code	3523 yellow code
187	3940 orange code	1225 orange code
325	3947 cream code	15 red code
331	2466 red code	3471 red code

Table 2. Effects of treatments (Non Treated, 50, 100 and 200 Gy) on the germination process (final germination percentage, FGP; first emergence, Em; half-time of germination, T_{50}; germination period, GPD; germination energy, Ge) and on the percentage of flowering seedlings (Fl).

Cross ID	**Treatment (Gy)**	**FGP (%)**	**Em (days)**	**T_{50} (days)**	**GPD (days)**	**Ge (%)**	**Fl (%)**
8	NT	32	60a[β]	67	81	28	50
	50	44	30b	67	81	34	68
	100	32	30b	74	81	18	37
	200	46	60a	74	81	34	52
	P	ns	*	ns	ns	ns	ns
51	NT	14	30b	74a	81a	8	86
	50	10	60a	74a	81a	6	100
	100	18	30b	60b	60b	8	100
	200	16	60a	60b	60b	10	75
	P	ns	*	*	**	ns	ns
72	NT	44	60a	67	81	22	68
	50	44	60a	67	81	32	59
	100	46	30b	74	81	32	57
	200	34	30b	74	81	18	53
	P	ns	*	ns	ns	ns	ns
187	NT	18	60a	67	81	14	67
	50	24	30b	67	81	20	75
	100	24	60a	67	81	18	75
	200	28	30b	67	81	22	86
	P	ns	*	ns	ns	ns	ns
325	NT	26	60a	60	60b	14	62
	50	36	30b	67	81a	18	67
	100	20	30b	60	81a	10	50
	200	40	60a	67	81a	24	45
	P	ns	*	ns	**	ns	ns
331	NT	14	60b	67b	81a	8	57
	50	8	30c	60b	81a	4	75
	100	14	60b	67b	60b	6	43
	200	2	81a	81a	81a	2	-
	P	ns	**	*	*	ns	ns

[β]Mean values followed by the same letter are not statistically different at $p \leq 0.05$ according to the REGW-F test. The statistical relevance of 'Between-Subjects Effects' tests (ns = non significant, * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.001$).

Table 3. Changes in percentage (%) of seed germination observed for each *Rosa hybrida* cross in comparison with the untreated sample (NT) at different radiation doses.

Dose	**Cross ID**					
	8	51	72	187	325	331
50 Gy	+12	- 4	0	+ 6	+10	- 6
100 Gy	0	+ 4	+ 2	+ 6	- 6	0
200 Gy	+ 14	+ 2	- 10	+ 10	+ 14	- 12