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Direct Shoot Regeneration in the Rose: Genetic Variation of Cultivars

Direkte Sproßregeneration bei Rosen: Genetische Variation der Sorten

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Summary

Genetic variation for direct shoot regeneration from *in vivo* grown leaf explants of 24 current cut rose cvs and clonal rootstocks was studied *in vitro*. Processes of shoot regeneration were followed for 25 days. Significant genetic variation occurred for: (i) percentage of shoot regeneration, (ii) days to shoot regeneration, (iii) number of adventitious buds and (iv) length of adventitious shoots. Significant correlations between these parameters occurred. Variation for (in)direct shoot regeneration in rosaceous crops is discussed. Present direct shoot regeneration is compared to vigour on plant level.

Zusammenfassung

Bei 24 gängigen Rosensorten und -unterlagen aus Gewächshauskultur wurde an *in vivo* Blattexplantaten die genetische Variation bei der direkten Sproßregeneration *in vitro* untersucht und für einen Zeitraum von 25 Tagen beobachtet. Signifikante genetische Variation trat für folgende Merkmale auf: 1. Prozentualer Anteil regenerierter Sprosse, 2. Tage bis zur Sproßregeneration, 3. Anzahl Adventivknospen und 4. Länge der Adventivsprosse. Zwischen diesen Parametern traten signifikante Korrelationen auf. Es wird die Variation für (in)direkte Sproßregeneration bei Rosaceae diskutiert. Die beobachtete direkte Sproßregeneration wird mit der Wuchskraft der Pflanze verglichen.

Introduction

In regeneration of the rose, embryogenesis rather than organogenesis has been pursued (DUBOIS and DE VRIES 1995, ROBERTS et al. 1995, VAN DER SALM 1996). In spite of that, first report of successful regeneration was on *in vitro* shoot organogenesis (HILL 1967). Attempts to repeat Hill's experiments yielded only 'promising' callus of a few cvs (KHOSH-KHUI and SINK 1982, VALLES and BOXUS 1987). Not until 1988 were adventitious shoots induced *in vitro* in several rose species (LLOYD et al. 1988) and in the Hybrid Tea 'Headliner' (ARIF and KATHAMIAN 1989). Adventitious bud-like structures of various *in vitro* Hybrid Tea cvs were reported by OLIVIER and CUSTERS (1988) and by BERARDI (1989), but these could not be induced to form shoots.

Indirect *in vitro* shoot regeneration was obtained by ISHIOKA and TANIMOTO (1990) from stem explants of *R. damascena* Miller, by BURGER et al. (1990) from callus of zygotic embryos of the Floribunda 'Bridal Pink', and from *in vitro* and *in vivo* grown leaf and shoot explants by ROUT et al. (1992) in the Hybrid Tea 'Landora', and from roots and leaves of 'Meirutral' by ARENE et al. (1993).

Direct shoot regeneration in the rose has been communicated twice, viz. from *in vitro* explants (LEFFRING and KOK 1990) and from *in vivo* explants (DUBOIS and DE VRIES 1995). Because direct shoot regeneration was successful in only few cvs and species, genetic variation for shoot regeneration could not be established. The present study reports on the differences as to direct shoot regeneration on leaf explants of 24 glasshouse-grown rose cvs incubated on media developed by DUBOIS and DE VRIES (1995).

Material and Methods

Plant material consisted of 24 cvs currently used as cut roses or as (clonal) rootstocks (Table 1). Plants of these cvs were grown in a heated glasshouse. Shoot tips (5–7 cm long) with partly folded leaflets were excised and disinfected in diluted (1% chlorine) technical bleach for maximal 5 mins. After 3 rinses (15 mins each) in autoclaved de-ionized water, leaves were cut from the shoot tips. Explants, consisting of the proximal half of a leaflet with its subtending petiolule, were incubated on induction medium in the dark at 24 °C for 8 days. Per cv 100 explants were incubated in 10 petri dishes, each containing 10 explants on 25 ml medium. After 8 days, explants were transferred to shooting medium and placed in the light. The experiment was ended 25 days after incubation.

Induction medium

- half-strength basal MS-salts and full strength MS-vitamins (MURASHIGE and SKOOG 1962)
- 30 g/L glucose
- 100 mg/L caseine hydrolysate
- 6.9 μM (1.5 mg/L) thidiazuron (TDZ)
- 0.49 μM (0.05 mg/L) indole-3-butyric acid (IBA)

adjusted to pH 5.6 before addition of:

- 8 g/L Daichin agar.

The medium was autoclaved at 120 °C for 20 mins.

- filter-sterilized $AgNO_3$ (60 μM, 10.0 mg/L) was added after autoclavation.

Shooting medium

– full-strength basal MS-salts and MS-vitamins (MURASHIGE and SKOOG 1962)
– 30 g/L glucose
– 2.2 μM (0.5 mg/L) benzylaminopurine (BAP)
– 0.05 μM (0.01 mg/L) IBA

adjusted to pH 5.8 before addition of:

– 8 g/L Daichin agar.

The medium was autoclaved at 120 °C for 20 mins.

– filter-sterilized GA_3 (0.3 μM) was added after autoclavation.

After transfer to shooting medium, petri dishes containing about 25 ml medium were placed at 24 °C in red light (8 μmol m^{-2} sec^{-1}) given by Thorn fluorecscent tubes, 16 h irradiation.

Experimental

An experimental unit consisted of 1 petri dish containing 10 explants. There were 10 blocks, each block containing 1 petri dish of each cv (24 dishes per block); within a block petri dishes were fully randomized. Statistical significance was determined at p = .05 with SPSS statistical package.

Observations

Records were made as to:

– the date of emergence of the first adventitious bud of an explant (binocular).

After 25 days:

– number of explants with at least 1 adventitious bud/shootlet
– number of adventitious buds/shootlets per explant
– length of the longest bud/shootlet of an explant (mm)

Definitions

– Percentage of shoot regeneration: the percentage of explants with at least one adventitious bud.

Table 1. The index number, commercial name and class (HT = Hybrid Tea, F = Floribunda, Min = Miniature, Can = *R. canina*, HMul = Hybrid multiflora) of 24 rose cvs tested for capacity of direct shoot regeneration.
Nummer, Name und Zuordnung der 24 Rosensorten, die auf die Fähigkeit zur direkten Sproßregeneration getestet wurden (HT = Tee-hybriden, F = Floribunda, Min = Zwergrosen, Can = Rosa canina, HMul = R. multiflora-Hybride)

Index #	CV name	Class	Index #	CV name	Class
1	Sonia	HT	17	Domingo	HT
2	Only Love	HT	18	Goldy	HT
4	Madelon	HT	20	Super Star	HT
5	Presto	HT	21	White Dream	F
6	Vicky Brown	HT	22	Moneyway	HMul
7	Landora	HT	23	Kuiper	Can
8	Melody	HT	26	Cara Mia	HT
9	Tineke	HT	29	Sprinter	HT
10	Frisco	F	168	Little Flake	Min
11	Vivaldi	HT	169	Souvenir	HT
14	Sturdu	Can	170	Cumbaja	F
16	Darling	HT	171	Lambada	F

Table 2. Between-cultivar correlations between (i) mean regeneration percentage, (ii) mean time to shoot regeneration, (iii) mean adventitious shoot length and (iv) mean number of adventitious buds per explant (n = 24 cvs, correlations significant at p = .05 (+) or .01 (++)).
Korrelationen zwischen Sorten, berechnet aus den Mittelwerten der Merkmale: 1. relative Regenerationsrate, 2. Zeit bis zur Sproßregeneration, 3. Adventivsproßlänge und 4. Anzahl Adventivknospen pro Explantat (n = 24 Sorten, Signifikanzniveau p = 0.05 (+) oder 0.01 (++)).

	Time	Length	Number
Regeneration%	–0.67++	0.47+	0.71++
Time	–	–0.47+	–0.62++
Length	–	–	0.87++

– Time to shoot regeneration: the number of days from incubation to emergence of the first adventitious bud per explant.

Results

Direct shoot regeneration in the present experiment followed a similar course to that described by DUBOIS and DE VRIES (1995). Infection did not occur. Depending on the time of emergence adventitious buds grew out into shootlets. New adventitious buds did not emerge beyond 25 days.

All cvs showed shoot regeneration. The visually large between-cultivar differences occurring for all parameters observed, were confirmed by statistical analysis. Figure 1 (top-left) shows that between cvs shoot regeneration varied from 62–100 %. According to Duncan's range test ($LSD_{.05}$ = 14.2 %), three significantly different groups of cvs could be distinguished viz. 'moderate' (cvs 23, 6, 14, 1), 'good' (cvs 29, 169, 171, 16, 5) or 'very good' (cvs 4, 18, 170, 7, 20, 168, 11, 26, 8, 10, 22, 9, 17, 21, 2). When arranged for increasing regeneration percentage, time to regeneration of cvs (Figure 1, top-right) decreased from approximately 18 to 12 days. The mean length of adventitious shoots of cvs (Figure 1, bottom-left), measured 25 days after incubation, generally increased as regeneration percentage was higher, viz. from approximately 1.0 to 8.0 mm. Likewise arranged, the number of adventitious buds of cvs (Figure 1, bottom-right) increased from approximately 0.5 to 4.0 per explant.

Significant correlations between parameters (Table 2) indicate that as the regeneration percentage of cvs was higher, (i) time to adventitious bud emergence was shorter, (ii) adventitious shoot length increased, (iii) more buds per explant emerged; as time to adventious bud emergence of cvs was longer, (iv) explants had shorter adventitious shoots, and (v) explants generated fewer buds; finally, as shoot length of cvs was longer, (vi) explants generated more adventitious buds.

Discussion

Variation for direct shoot regeneration was studied in a representative sample of 24 rose cvs, covering various classes of (glasshouse) roses. Leaf explants of these cvs, obtained from glasshouse-grown plants, formed adventitious buds between 62 and 100 %, in 12 to 18 days.

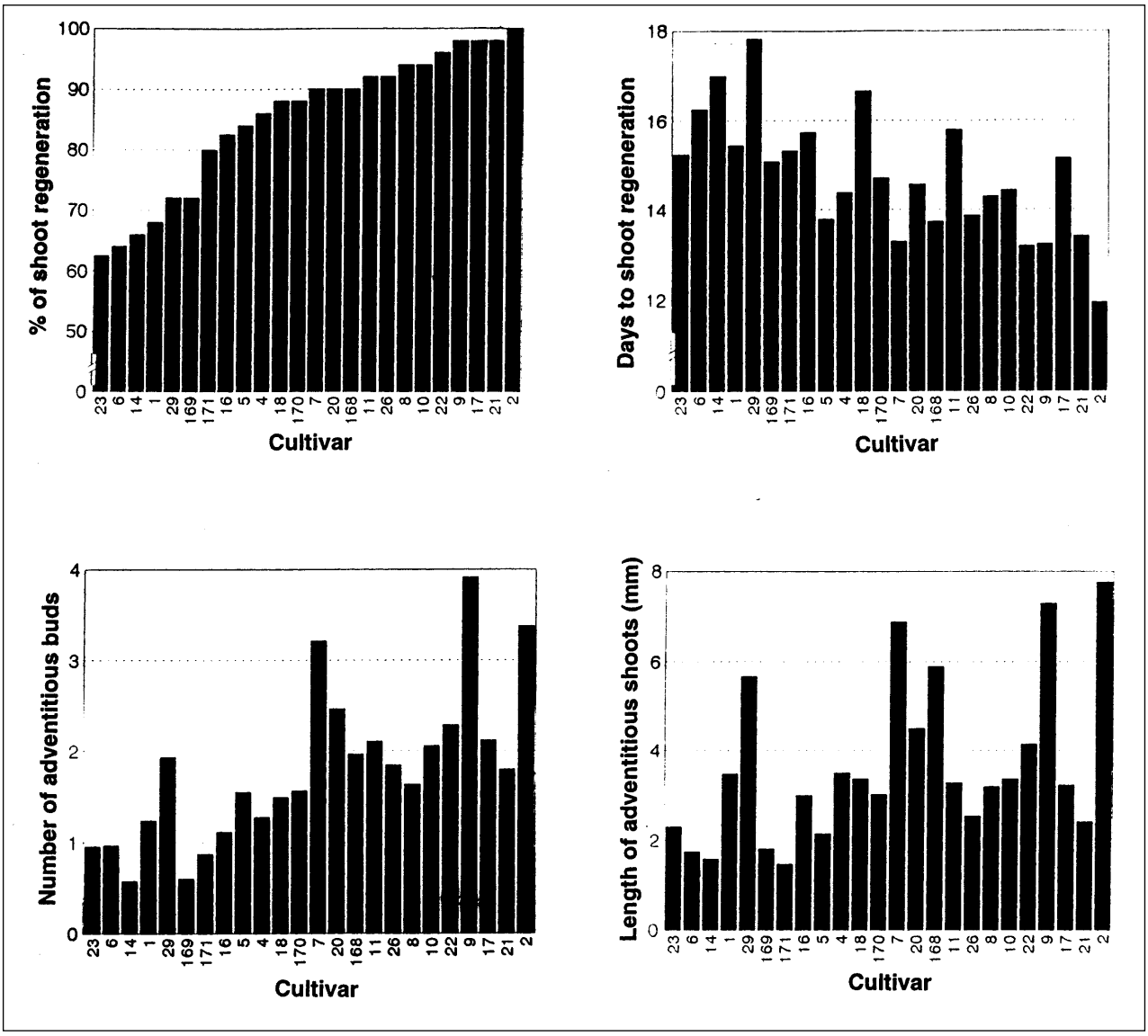


Figure 1. The percentage of direct regeneration (top left), the number of days to shoot regeneration (top right), the number of adventitious buds per regenerating explant (bottom left) and the length of adventitious shoots (bottom right), of 24 rose cvs. For each parameter cvs are arranged for increasing percentage of regeneration.
Prozentualer Anteil direkter Regeneration (oben links), Anzahl der Tage bis zur Sproßregeneration (oben rechts), Anzahl Adventivknospen/Explantat (unten links) und Länge der Adventivsprosse (unten rechts) von 24 Rosensorten. Die Sorten wurden für jeden Parameter nach zunehmender relativer Regeneration sortiert.

Significant genotypic variation occurred for the parameters: regeneration percentage, time to shoot regeneration, number of adventitious buds per explant and adventitious shoot length. These results confirm earlier presumptions (DUBOIS and DE VRIES 1995).

As to the percentage of regeneration, cvs could be divided into three significantly different groups, arbitrarily indicated as moderate, good and very good. In practice, however, differences between these cvs were negligibly small. It is concluded, therefore, that contrary to earlier opinion (VALLES and BOXUS 1987) roses are not very recalcitrant to direct shoot regeneration.

(In)direct shoot regeneration, mainly aiming at a suitable system of genetic modification, has been studied in several rosaceous crops. In the majority of experiments, however, only few genotypes were studied. In those with two or more genotypes, emphasis was on plant tissue culture media rather than on genetic variation. Indications are, however, that cvs of blackberry (HASSAN et al. 1993), raspberry (COUSINEAU and DONELLY 1991), strawberry (SORVARI et al. 1993), apricot, domestic plum (ESCALLETTES and DOSBA 1993), and particularly those of apple (KORBAN et al. 1992; FASOLO et al. 1989; JAMES et al. 1988) and pear (CHEVREAU et al. 1989), show genetic variation for shoot regeneration. As authors commonly report frequencies between 0 and 100%, present results indicate that roses of which cvs regenerated for at least 65%, probably form a favourable exception to that rule.

Inter-cultivar correlations show that as regeneration percentage of cvs was higher, time to shoot regenera-

tion was shorter, while explants generated more and longer adventitious buds. It is only speculative which of these parameters are 'independent', and which 'dependent'. If regeneration capacity would depend on sensitivity of tissue to cytokinins, it may be reasoned that as sensitivity of genotypes increases, more explants would form more adventitious buds in a shorter time. As a consequence, these buds would emerge earlier and hence be longer at the same moment. On the other hand, genetic variation for the number of 'target cells' (MARGARA 1982) which may explain variation for the number of adventitious shoots, cannot be excluded. Variation for the number of target cells, however, seems not to explain variation for the time of emergence of adventitious buds. It is presently being investigated whether or not there is a relation between the frequencies of shoot regeneration and of successful *Agrobacterium* transformation.

As to the efficiency of direct regeneration from leaf explants a difference of opinion has occurred as to the use of *in vitro* or *in vivo* sources. Laboratories using *in vitro* explants, incubate a complete leaf composed of 3–7 leaflets on induction medium. Such explants may yield 2–6 adventitious shoots (DUBOIS, unpublished results). In the present experiment the mean number of adventitious shoots per explant, prepared from one (*in vivo*) leaflet only, was 1.86. Consequently, one *in vivo* leaf (divided into 3–7 leaflets) would have yielded 5.6–13.0 adventitious shoots. This indicates that in addition to being much cheaper (DUBOIS and DE VRIES 1995), the *in vivo* method is more efficient than the *in vitro* method.

Comparison between aspects of growth and development *in planta* and that of the same cvs *in vitro*, has hitherto been hampered due to lack of data. It was tempting, therefore, to interprete present percentages of regeneration in relation to vigour on plant level. It is notable that the important parameter of vigour 'flower yield' of cut rose cvs, is directly associated with their 'branching capacity'. As branching capacity, in turn, is controlled by the ratio of (endogenous) auxins: cytokinins (DE VRIES 1993), a connection with regeneration capacity, which likewise depends on hormone ratios, cannot be excluded. On the basis of flowers m^{-2} $year^{-1}$ published for 11 of present cvs (BOERMA and VAN RIJBROEK 1994), linear regression analysis was carried out. Because correlation (r) between yield and regeneration percentage was 0.10 (n = 11; p .05 is significant at r = .60), it is concluded that there is no indication whatsoever that capacity of direct shoot regeneration of the cvs involved is related to yield.

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