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INTERACTION BETWEEN IN VITRO SHOOT FORMING CAPACITY AND X-RAY SENSITIVITY OF *GERBERA JAMESONII, BOLUS*

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A b s t r a c t. There is not only a broad variation concerning in vitro shoot forming capacity among various idiotypes of Gerbera jamesonii, but there also exist differences in radiosensitivity measurable by the X-ray dose dependent inhibition of shoot development. Investigations on the existence of a correlative interaction between 'high shoot forming capacity' and 'low radiosensitivity' or vice versa did not result in clear cut findings, because up to four weeks post-irradiation no influence of shoot forming capacity could be measured. On later dates, however, the influence of shoot forming capacity became more and more significant. This led to the decision to use only the number of shoots on the first date of cut-off to determine radiosensitivity and to fix the so-called 'optimum-dose' for in vitro mutation induction experiments.

INTRODUCTION

The test of radiosensitivity is considered a prerequisite for inclusion of mutation induction experiments in breeding programmes [1,2,3]. For plant species propagatable by *in vitro* derived axillary shoots (*Gerbera, Rosa, Prunus, Pelargonium etc.*) the X-ray dose dependent inhibition of shoot development can be utilized to estimate radiation damage as well as to determine the so-called 'optimum-dose' [1].

There are genetically fixed differences with regard to the shoot forming capacity (SFC) among various idiotypes of *Gerbera jamesonii* and it was supposed to be some influence of regeneration rate either on radiosensitivity and/or on the speed of the recovery from radiation damage.

It was the objective of the study to investigate whether there exists any correlative interrelation between shoot forming capacity and X-ray sensitivity.

MATERIALS AND METHODS

Five lines of *Gerbera jam.* developed in the BFA Ahrensburg and one cultivar were included in the experiments (Table 1).

The idiotypes in Table 1 are arranged on the basis of decreasing SFC on the first date of cut-off. Pre- and post-irradiation conditions as well as the procedure for X-irradiation were described earlier [4]. The technical data of the X-ray machine were: 12 mA, 150 kV, 1.7 mm Al-filter; dose rate: 0.9 Gy min^{-1}. The number of post-irradiation produced axillary shoots was determined on four successive dates of shoot detachment in intervals of four weeks. The cumulative number of shoots was obtained by addition of the average number of shoots produced by 10 explants on the subsequent dates. The number of replications was 3-9; one replication consisted of 10 explants per Petri dish.

Table 1. Description of Gerbera idiotypes

Idiotypes No.	Idiotypes Name	Plant type	Flower colour	Colour of the centre of the flower ("eye")
1	BFA 84/115-1	pot	pink	light
2	BFA 85/382-3	pot	lilac	dark
3	BFA 84/131-13	pot	yellow	light
4	BFA A 26	pot	cherry red	light
5	Clementine	pot	orange	light
6	BFA 82/19-6	pot	pink	light

RESULTS

Shoot developing capacity of various idiotypes of *Gerbera jamesonii*

The well known fact that *in vitro* regeneration rates among *Gerbera* idiotypes are very different is also illustrated by the curves for control in Fig. 1. The idiotypes are arranged from left to right on the basis of the average number of shoots developed by 10 explants during the first 4 weeks of *in vitro* culture and this is the same sequence as in Table 1. Striking differences are documented, but also the tremendous increase of shoot production on the following three dates of cutoff. There seems to be a relationship between the number of shoots developed on the first date of cut-off and the total production during 16 weeks ($r=0.7824$). Such a profile is certain of interest for mutation induction experiments as described in the next chapter.

Radiosensitivity of various idiotypes of *Gerbera jamesonii*

The first date of cut-off axillary shoots was 4 weeks post-irradiation. This period was chosen, because at this time the shoots of the unirradiated explants (control) were ready for detachment. Consequently, any shortage of shoot production on this date was considered X-ray dose dependent as well as influenced by the genetically fixed radiosensitivity. In this connection the general effect of the X-ray treatment on the propagation profile of *in vitro* derived microshoots may be of some interest. Calculating the relative shoot productivity on each of the subsequent dates of cut-off (total production per variant = 100 %) results in distribution designs demonstrated in Fig. 1.

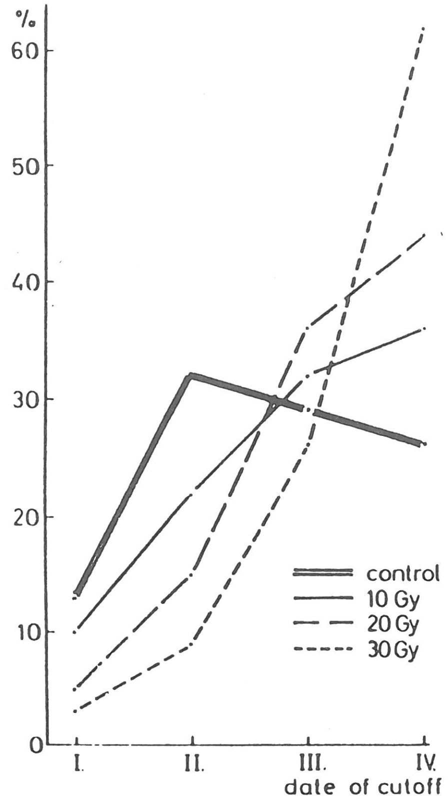

Fig. 1. Relative shoot production of Gerbera on 4 subsequent dates of cut-off. All values were transformed by addition of 1, because on the 1st date of cut-off - 30 Gy dose - the number of developed shoots was zero [5].

The shoot development of the control is lowest on the first date and increases during the second period of cultivation and later it becomes less. The design of the profile changes as the X-ray doses enlarge, i.e., the maximum production is shifted to later dates of cut-off in comparison with that of the control.

In Fig. 2 the idiotypes are arranged on basis of the relative shoot production on the first date of shoot detachment by an X-ray dose of 10 Gy.

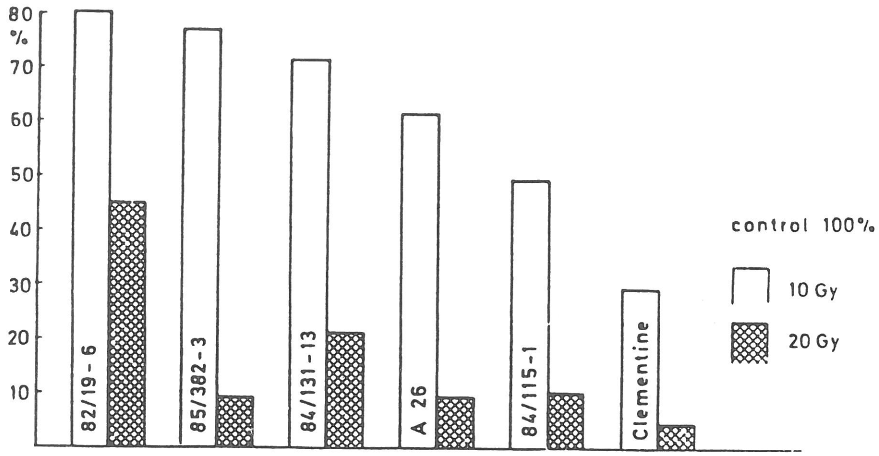

Fig. 2. Relative shoot production on the 1st date of cut-off by doses of 10 Gy and 20 Gy.

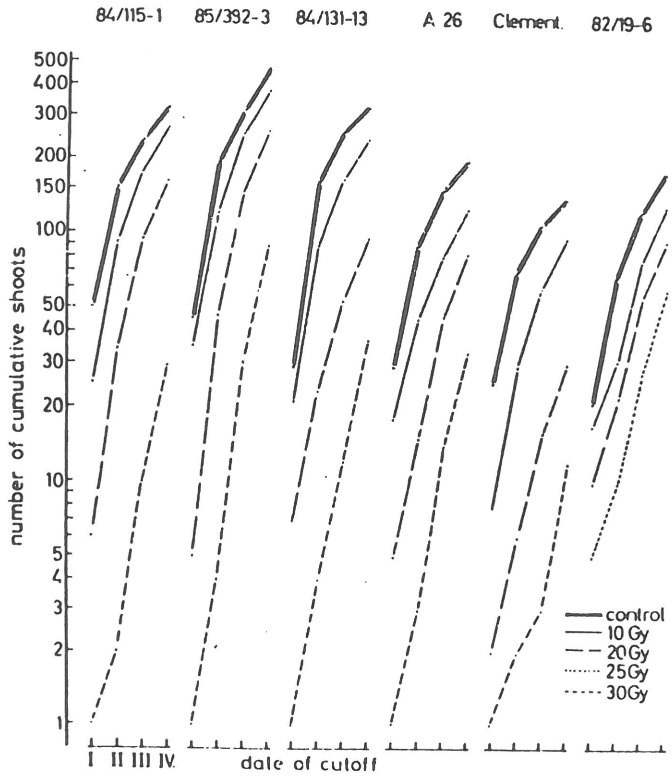

Fig. 3. Radiosensitivity of various idiotypes of Gerbera jam. All values were transformed by addition of 1, because on the 1st date of cut off - 30 Gy dose - the number of developed shoots was zero [5].

Table 2. Correlation coefficients basing on the cumulative numbers of shoots on 4 subsequent dates of cut-off

Date of cut-off	Control 10 Gy	Control 20 Gy	Control 30 Gy*
I	0.7800	-0.1839	—
I + II	0.9954+++	0.8419+	0.5894
I + II + III	0.9756+++	0.8389+	0.7720
I + II + III + IV	0.9942+++	0.9141+	0.8740

* Only 5 idiotypes were involved in this irradiation experiment; + significant at P < 5 %; ++ significant at P < 1 %; +++ significant at P < 0.1 %.

The sequence of the idiotypes is different to that in Table 1 indicating only a weak relationship between SFC and radiosensitivity on this date as a result of a dose of 10 Gy.

The influence of different X-ray doses on the development of axillary shoots during a period of 16 weeks, i.e., on 4 subsequent dates of cut-off, is demonstrated by the propagation profiles in Fig. 3. The idiotypes are arranged as in Table 1 and the curves were constructed by using the average cumulative numbers of shoots (10 explants).

Interaction between shoot forming capacity and radiosensitivity

On the basis of the cumulative number of axillary shoots the coefficients of correlation between SFC of the control and that of the different X-ray dose variants were calculated and the results are presented in Table 2.

For determination of the typical radiosensitivity of each idiotype Walther and Sauer [1] recommended to use the number of shoots produced on the first date of cut-off. Obviously, on this date no correlation can be stated between shoot developing capacity and radiosensitivity. Comparing the correlation coefficients calculated for the variants 'control-10 Gy' to those including control and doses higher than 10 Gy demonstrates the decrease of statistical significance of the correlation coefficients with increasing doses.

Similar results were found calculating correlation coefficients on the basis of the absolute number of shoots removed on each date of cut-off (Table 3).

Table 3. Correlation coefficients basing on the absolute numbers of shoots produced on 4 subsequent dates of cut-off

Date of cut-off	Control 10 Gy	Control 20 Gy	Control 30 Gy*
I	0.7800	-0.1839	-
II	0.9752++	0.8134+	0.7215
III	0.9417+	0.8424+	0.7968
IV	0.9880++	0.9927++	0.9262+

* Only 5 idiotypes were included in this experiment.

From the data in both these tables basically the same tendency can be recognized: there is no correlation on the first date of cut-off, but on later dates the dose dependent decreased production is influenced by the idiotypically SFC.

DISCUSSION AND CONCLUSIONS

A small sample size out of a large number of idiotypes available was included in the experiments, but the results described above are considered typical for propagation of *Gerbera jamesonii* by *in vitro* derived microshoots.

The number of developed shoots per 10 explants varied between 452 and 138 for a period of 16 weeks of *in vitro* culture. In the same time period the X-ray dose reduced production was 301-199 shoots as the result of a 10 Gy dose, 136-84 shoots after the 20 Gy treatment and 34-14 as consequence of the highest dose of 30 Gy. The broad variation of SFC in the control and a similar tendency in the X-irradiated explants led to the assumption that there seems to be a relationship between both these characteristics as can be concluded from the graph in Fig. 3.

A correlative interaction was found in case of all X-ray dose variants on the second and later dates of shoot detachment, especially concerning the total shoot production up to 16 weeks. There was no statistical significance, however, on the first date of shoot removal. This indicates the strong influence of the radiosensitivity expressed by differences in regeneration rates. The weak correlative interaction between control and 10 Gy variant on the first date may be the result of the limited damage induced by that relativly low dose only partly masking the SFC. This observation is also supported by the design of the curve for 10 Gy in Fig. 1.

To what extent the SFC of the various idiotypes is responsible for differences in radiosensitivity cannot be stated on the basis of these results. In the case of the application of relative low doses the influence of 'high' or 'low' shoot yielding idiotypes on radiosensitivity is observable up to the first date of cut-off. The positive correlation coefficients calculated for the interaction between SFC of the control and that of X-rayed explants pointed to the strong influence of the typical shoot productivity of the idiotypes after recovery from a certain amount of radiation damage during the first post-irradiation period. For mutation induction experiments the results described above support the recommendations made earlier: tests of radiosensitivity are a necessity.

Gerbera jamesonii may be considered a reference system serving as a model for those plant species propagatable by axillary shoots under *in vitro* culture conditions.

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