Theor Appl Genet (1985) 70:128–132

A comparative study of genotypic and environmental response to androgenesis in *Nicotiana rustica*

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Received September 20, 1984
Communicated by R. Riley

Summary. A total of six genotypes of *Nicotiana rustica* comprising the two F_1's ($V_2 \times V_{12}$ and $V_1 \times V_5$) and their parents were evaluated for their efficiency in haploid production. Excised immature flower buds with pollen at late uninucleate to early binucleate stage were pretreated for 21 days at 5° or 7 °C, or for 15 days at 9 °C before culturing on Nitsch's medium + 0.1 mg/l NAA. The effects of genotype, pretreatment and their interaction were tested on anther response, anther productivity and days to first plantlet formation. Highly significant genotype × pretreatment interaction and differences between genotypes were observed for all three characters. Significant differences between pretreatments were observed for anther productivity only. The performance of V_{12} both in respect of anther productivity and response was highest whereas that of V_5 was the lowest. Analysis of variance showed that a simple additive genetic model was not adequate to explain the above variation due to significant additive genetic and dominance interactions with the pretreatment.

Key words: Anther culture – Androgenesis – Haploid production – Pretreatment – Tobacco

Introduction

Following the report of Guha and Maheshwari (1964, 1966) that haploid embryoids could be easily produced from the pollen grains of *Datura innoxia* through anther culture, this method was tried in a large number of species in attempts to develop rapid inbreeding programmes for crop improvement. The haploid technique may be compared with conventional breeding methods only when the production of a large number of haploids is possible. To maximise haploid production many treatments have been tried, the most successful being temperature-pretreatment (Sunderland and Roberts 1979; Keller and Armstrong 1979; Genovesi and Collins 1982; Huang and Sunderland 1982; Chowdhury 1984b).

An extensive investigation to find the best combination of pretreatment in *Nicotiana tabacum* has been carried out by Sunderland and Roberts (1979) who reported that temperatures below 7 °C are only marginally effective and are deleterious. On the other hand, Chowdhury (1984b) has recently shown that in *N. rustica*, temperatures below 7 °C when given for a longer duration are also effective, although to a lesser extent. The optimum temperature and duration of pretreatment varies from genotype to genotype (Sunderland 1980; Genovesi and Collins 1982).

Recently, Foroughi-Wehr et al. (1982) showed the great importance of genetic factors in in vitro culture of barley anthers. These workers indicated that androgenesis ability is heritable and therefore can be transferred to non-responsive materials which are superior in important agronomic characters. In order to find the most responsive material it is necessary to screen a large number of genotypes of a species or its close relatives because only a few genotypes in a given species may respond to anther culture. Gresshoff and Doy (1972), for example, found that only one out of 43 tomato genotypes responded to haploid production. Most of the investigations to improve anther response involved a test of either a number of environmental factors on one genotype (Dunwell 1976; Sunderland 1978) or a number of genotypes under one environmental condition (Gresshoff and Doy 1972; Islam et al. 1980; Foroughi-Wehr et al. 1982). Recently, the scope of investigation has been widened to include a number of genotypes under different environmental conditions (Lazar et al. 1984) as well as the effect of media and cold shock (Genovesi and Collins 1982). As a result of such an investigation significant genotype × pretreatment (G × P) and differences among genotypes, media and cold shock emerged.

Since work of this kind has not been undertaken on *N. rustica*, it was considered worthwhile to study the effect of three cold pretreatments on six genotypes for

their anther response (AR), anther productivity (AP) and days to first plantlet formation (DFPF). The study involved a number of aspects namely; 1) whether certain genotypes respond equally well under all pretreatment conditions; 2) whether all genotypes respond equally at a particular pretreatmet; 3) whether genotype × pretreatment (G × P) interaction is important and 4) the mode of gene action of the characters studied.

Materials and methods

Varieties 1 and 5 of *N. rustica* and their F_1 (cross 1), and varieties 2 and 12 and their F_1 (cross 2), were used. Ten plants from each of the six genotypes were raised in the greenhouse under a 16 h day length at 20 °C. For pretreatments, flower buds with corolla lengths of between 3.5 and 5.0 mm (stages 3–5 confirmed by cytological observations as described by Chowdhury 1984b) from each genotype were collected and placed in plastic petri dishes. To maintain the humidity, a few drops of water were placed in each dish which was then sealed with nescofilm. The length of petals was measured to determine the developmental stages of the pollen grains. Water loss was minimised by wrapping each dish in aluminium foil and storing it in either a refrigerator at 5 ± 0.5 °C for 21 days (pretreatment 1) or a refrigerated incubator at 7 ± 0.5 °C for 21 days (pretreatment 2) and at 9 ± 0.5 °C for 15 days (pretreatment 3).

All the five anthers from a pretreated flower bud were cultured on 5 ml of Nitsch (1969) + 0.1 mg/l NAA medium in a plastic petri dish, sealed with nescofilm and incubated inside a growth cabinet under 18/6 h light regime at 24 °C/20 °C, respectively. Culture procedures and incubation methods were the same as described previously (Chowdhury 1984b). Three hundred anthers from each genotype (100 from each pretreatment) were cultured and all 18 combinations were completely randomised in the growth cabinet. Both the main effects (genotype and pretreatment) were considered fixed, in the statistical sense.

DFPF was recorded as the number of days from the date of anther culture to the first plantlet formation. AP was estimated as the total number of plantlets divided by the total number of anthers cultured. AR was measured as the number of anthers that produced plantlets divided by the total number of anthers cultured, times 100.

For AR individual anthers were scored as either responding or not responding, which are mutually exclusive. These data are not normally distributed and the sampling errors are not independent of the observations. To analyse these data using Analysis of Variance it was necessary to transform them so that the errors become independent of observations. This was done by the angular transformation, $P = \mathrm{Sin}^2\theta$, where P is the proportion varying from 0–1 and θ is an angle varying from 0–90 degrees. In large samples (n = 100 in the present case) θ tends to be normally distributed and has a variance which is dependent on n only, because $\sigma^2\theta = 820.7/n$ (a theoretical error).

Results

Highly significant differences between genotypes and G × P interaction were observed for all the three characters scored (Table 1). Differences between pretreatments for AR and DFPF were not significant but for AP were highly significant (Table 1). The appropriate error for testing the main effects for AP and DFPF was the within genotype within pretreatment variance but for AR the G × P variance (significantly different from theoretical error) was used because the within genotype within pretreatment variance was a theoretical error.

Table 1. Analysis of variance of anther response, anther productivity and days to first plantlet formation of six genotypes at three temperature pretreatments

Item	df	MS		
		AR	AP	DFPF
Between genotypes	5	460.23***	2.30***	206.86**
Between parents (cross 1)	1	177.13*	0.1634*	650.34***
Between parents (cross 2)	1	698.33***	4.05***	223.04**
Between crosses	1	1,355.3***	7.22***	66.58*
Between mid-parent (MP) and F_1 (cross 1)	1	64.45 NS	0.037 NS	71.65*
Between MP and F_1 (cross 2)	1	5.63 NS	0.034 NS	22.67 NS
Between pretreatments	2	18.94 NS	0.4326***	40.63 NS
Genotype × pretreatments	10	31.42***	0.4345***	56.0***
Between parents × pretreatments (cross 1)	2	0.246 NS	0.00065 NS	22.957 NS
Between parents × pretreatments (cross 2)	2	61.8***	1.0122***	173.524***
Between crosses × pretreatments	2	8.665 NS	0.3804***	14.95 NS
(Between MP and F_1) × pretreatments	2	0.785 NS	0.0024 NS	44.14*
(Between MP and F_1) × pretreatments	2	85.64***	0.779***	24.32 NS
Within genotypes within pretreatment	α, 1782 and 506	8.207	0.034	14.33

*, **, *** Significant difference at $P = 0.05$, 0.01 and 0.001, respectively
NS = non-significant difference

The between genotype sum of squares (SS) for each character were partitioned into between parents within crosses (cross 1 and 2), between genotypes (parents and F_1) between crosses and between mid-parental (MP) value and F_1 mean within crosses (cross 1 and 2). For both crosses and all the three characters the parental difference, which is ascribable to the additive genetic component [d] (Mather and Jinks 1971) was significant although for AR and AP the differences were less significant in cross 1 than in cross 2 and the DFPF parental difference was less significant in cross 2 than in cross 1 (Table 1). The difference between the two crosses was highly significant for AR and AP and marginally significant for DFPF. No significant difference between MP value and F_1 mean was observed for AR and AP in either case hence there are no significant dominance deviations, [h], (Mather and Jinks 1971) for these characters.

The genotype × pretreatment SS for each character were also partitioned to correspond with the partitioning of the genotypes SS into between parents within crosses × pretreatment (cross 1 and 2), between genotypes between crosses × pretreatment and between mid-parental value and F_1 mean within crosses × pretreatment (cross 1 and 2). Highly significant between parents × pretreatment interaction which is ascribable to the additive genetic × pretreatment interaction component g_d (Mather and Jinks 1971) was observed for all characters in cross 2 but not in cross 1 (Table 1). Between crosses × pretreatment was highly significant for AP but non-significant for AR and DFPF. Between mid-parental value and F_1 mean × pretreatment, which is ascribable to the dominance deviation × pretreatment g_h (Mather and Jinks 1971) was marginally significant for DFPF in the cross 1 and highly significant for AR and AP in the cross 2 (Table 1).

In respect of AR and AP, V_{12} was the most responsive and V_5 the least (Tables 2 and 3). V_5 also took longer to produce the first plantlets (Table 4). The least responsive genotype (V_2) from cross 2 was better than the best (V_1) from cross 1.

Significant differences on Duncan's New Multiple Range Test (Tables 2 and 3) were observed between the genotypes for anther response and between genotypes and pretreatments for anther productivity.

Pairwise correlations between the three characters over the 6 genotypes showed a highly significant positive correlation (0.95) between AR and AP and non-significant negative correlations between AR and DFPF (–0.42) and between AP and DFPF (–0.32).

Table 2. Percentage of anthers responding to produce plantlets from anther culture

Pretreatment / Genotype	21 days at 5 °C (1)	21 days at 7 °C (2)	15 days at 9 °C (3)	Genotype mean
V_1	22	18	20	20.0 b c
V_5	8	6	8	7.33 c
$V_1 \times V_5$	21	20	20	20.33 b c
V_2	30	21	20	23.67 b c
V_{12}	64	39	75	59.33 a
$V_2 \times V_{12}$	32	53	47	44.0 a b
Pretreatment mean	29.5	26.16	31.67	

Means with the same letter are not significantly different at $P = 0.05$ according to Duncan's New Multiple Range Test

Discussion

There are three principal ways of improving anther culture response, by a) changing the physiological status of the donor plants e.g.

1) growing the plants under different photoperiodic conditions (Dunwell 1976);

Table 3. Anther productivity by main effects (genotypes and temperature-pretreatments) for plantlet production

Pretreatment / Genotype	21 days at 5 °C (1)		21 days at 7 °C (2)		15 days at 9 °C (3)		Genotype mean
V_1	0.46 c	C	0.41 b c	C	0.50 c	C	0.457
V_5	0.14 c	C	0.11 c	C	0.13 c	C	0.127
$V_1 \times V_5$	0.48 c	C	0.42 b c	C	0.383 c	C	0.428
V_2	1.30 b	B	0.43 b c	C	0.48 c	C	0.737
V_{12}	2.65 a	B	0.82 b	C	3.67 a	A	2.38
$V_2 \times V_{12}$	1.10 b	B	1.90 a	A	2.08 b	A	1.693
Pretreatment mean	1.02		0.68		1.21		

Means with the same letter (small for genotypes and capital for pretreatments) are not significantly different at $P = 0.05$ according to Duncan's New Multiple Range Test

Table 4. Duration by main effects (genotypes and temperature-pretreatment) for days to first plantlet production

Pretreatment / Genotype	21 days at 5 °C (1)	21 days at 7 °C (2)	15 days at 9 °C (3)	Genotype mean
V_1	20.29	21.7	19.63	20.49
V_5	27.3	29.62	24.32	26.85
$V_1 \times V_5$	22.8	23.52	24.61	23.63
V_2	18.85	20.5	22.38	20.33
V_{12}	23.46	23.33	21.08	22.43
$V_2 \times V_{12}$	23.67	22.14	21.67	22.34
Pretreatment mean	22.73	23.47	22.28	

2) nitrogen starvation (Sunderland 1978);
3) temperature-pretreatment of excised flower buds (Sunderland and Roberts 1979; Chowdhury 1984b) or inflorescences (Huang and Sunderland 1982).

b) the adjustment of cultural conditions, e.g. either by changing media composition for culturing of anthers, or light or temperature, during culture period and c) the genetic improvement of the donor plant. Since only one medium for culturing and uniform light and temperature throughout the culture period were used in this investigation it does not allow one to judge whether adjustment of cultural conditions could improve the anther response. Manipulation of pretreatment (Huang and Sunderland 1982) and/or transfer of gene(s) responsible for high androgenic potential to a low responding material (Wenzel et al. 1977; Mitchell et al. 1980) may, however, increase the response and productivity of a given genotype.

There are highly significant differences between genotypes for all the three characters. Genotypic differences for AR have also been observed by other workers, e.g. Gresshoff and Doy (1972) in tomato, Wenzel et al. (1977) in rye, Jacobsen and Sopory (1978) in potato, Islam et al. (1980) in rice, Genovesi and Collins (1982) in maize, Foroughi-Wehr et al. (1982) in barley, Lazer et al. (1984) in wheat and Chowdhury (1984a) in tobacco. Dunwell (1978) has suggested that such differences in androgenic response may result from differences in specific endogenous amino acids which allow embryogenic induction. This explanation has also been supported by Sangwan (1978) who observed that in *Datura metel* about 71% of the total free amino acid pool consists of threonine, serine, glutamic acid, proline and γ-aminobutyric acid during the induction phase of pollen embryoid formation.

For AP highly significant G×P interaction and differences for both main effects (genotype and pretreatment) were observed. Similar results were also observed by Genovesi and Collins (1982) in maize and Lazar et al. (1984) in wheat. The above findings clearly indicate that 1) the optimum conditions for plantlet formation varies from genotype to genotype; 2) different genotypes respond differently to different pretreatments. Information of this kind may help breeders choose the best genotypes for breeding by dihaploidy.

Partitioning of the total between genotype SS for all the three characters showed 1) significant parental differences within and between crosses, indicating the presence of additive gene action. This suggests that the transfer of androgenic response from a higher responding to a lower responding genotype will be possible. The importance of the genetic factor in this investigation is exhibited clearly in that under the best pretreatment the highest yielding genotype yielded approximately 28 times more than the lowest yielding genotype. The above finding also suggests that for breeding purposes a search for a genetically high responding genotype associated with high yield may be of more value than the other two methods. However, if high yield is not associated with high responsiveness for haploid production, one could always try to transfer responsiveness to the non-responsive but high yielding genotype. 2) Lack of a significant difference between MP and F_1 mean for AR and AP suggests the absence of net directional dominance ([h]=0). High positive heterosis observed in cross 2 for AR and AP is due to dominance deviation×pretreatment interaction (g_h positive). The highly significant G×P interaction indicates that the determination of suitable pretreatment condition may further increase the responsiveness and productivity of both the genetically high and low responsive genotypes.

On average over all genotypes pretreatment 3 gave the highest yield and the earliest response. This superiority of pretreatment 3 for the cross $V_2 \times V_{12}$ has been reported earlier by Chowdhury (1984b). The difference between pretreatment 3 and 1, however, is not very large and with limited facilities pretreatment 1 may be more convenient.

Since the two characters, AR and AP, are similar and highly correlated, these two traits are either the pleiotropic expression of the same gene(s) or they are controlled by genes in linkage disequilibrium. In the present investigation these alternative explanations cannot be separated. A similar high correlation between regeneration frequency and multiple embryoid formation was also observed by Lazar et al. (1984) in wheat. Non-significant negative correlations between DFPF and AR or AP indicate that they are under the control of independent genes.

Acknowledgements. I would like to thank Professor J. L. Jinks and Dr. H. S. Pooni for their guidance throughout the work; Dr. M. A. Cornish of this Department and Professor A. S. Islam, Dhaka University, Bangladesh (visiting Professor at Nottingham University on an academic link programme) for their constructive criticism and suggestions during the manuscript preparation. I am also grateful to the Commonwealth Scholarship Commission for financial assistance.

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