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Genetic factors influencing regeneration ability in rye (*Secale cereale* L.). I. Immature inflorescences

M. Rakoczy-Trojanowska, S. Malepszy

Institute of Horticultural Plant Genetics and Breeding, Warsaw Agricultural University, 02-766 Warszawa, Nowoursynowska 166, Poland

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Abstract. Immature inflorescences of ten rye inbred lines (inbred degree S10 and S11) were cultured on solidified MS medium supplemented with 3.0 mg/dm^3 of 2,4-D. According to their capability for callus production explants were classified into two groups: responsive (giving weak or intensive callus production) and non responsive (lack of callus formation). After transferring responsive material into hormone-free medium the regeneration of roots or shoots from the intensive growing callus was observed. Consistent differences between lines in the portion of explants with a certain response were found. They were divided into five groups reacting in the same way. Lines with different in-vitro response were crossed in an incomplete diallel. F_1, F_2 and F_3 generations were analyzed and the following conclusions drawn: the ability for plant regeneration from immature inflorescences in rye is determined by numerous loci, has a recessive character, and both callus production and regeneration suppression may be controlled by complementary genes.

Key words: Rye – *Secale cereale* L. – Immature inflorescence – Regeneration ability – In-vitro response

Introduction

The dynamic development of biotechnological methods in cereals requires a recognition of the genetic background capable of facilitating plant regeneration. For many cereals like maize (Thomas and Scott 1985), barley (Hanzel et al. 1985), wheat (Mathias and Fukui 1986; Lazar et al. 1987; Kaleikau et al. 1989a, b), rice (Toriyama et al. 1986), and rye (Krumbiegel-Schroeren et al. 1984; Rybczynski and Zimny 1985; Linacero and Vazquez 1986, 1990) regeneration ability has proved to be dependent on the genotype. However, the genetic factors responsible for this trait have been defined in only a few cases, mainly in wheat (Galiba et al. 1986; Lazar et al. 1987; Szakacs et al. 1988; Kaleikau et al. 1989a, b). The aim of our paper is to present a genetical analysis of plant (root) regeneration capability and the ability for callus production by immature inflorescences of rye. It is the first step in a wide range of experiments dealing with the regeneration ability of different explants, in which work on regeneration from immature embryos is currently the most advanced

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Correspondence to: M. Rakoczy-Trojanowska

Materials and methods

Ten inbred lines of rye, designated DW28, H363, L318, D855, H32, Pw330, L9, L29, L299 and H316, were used as a source of explants. All lines have been developed in our department. The main experiments were done between 1987 and 1991. Lines were screened in two seasons, during 1987, 1988, and F_1, F_2 and F_3 generations in the next two seasons. The following crosses were then made: Dw28 × H363, H363 × L318, D855 × L318, H32 × Pw330, L9 × L29 and L299 × H316.

Each plant was represented by one inflorescence (explant) and 1–2 cm-long explants were collected from the first, or occasionally the second, shoot. Shoots were cut into 4–6 cm segments and sterilized with 70% ethanol for 30 s, followed by sterilization in 7% sodium perchlorate, and then rinsed three times with sterile water. Explants were isolated under sterile conditions and placed on Petri dishes – ten explants per dish – with solidified medium. The medium used for callus induction was MS (Murashige and Skoog 1962) with 3.0 mg/dm^3 2,4-D, 20.0 g/dm^3 sucrose and 7.5 g/dm^3 Difco agar. Explants or calli, if possible, were subcultured every 4 weeks. After three subcultures they were placed into a regenerating medium – the same as used for callus induction but without hormones. Plantlets were transferred into a half-strength hormone-free MS medium with 15.0 g/dm^3 sucrose and 1.5 mg/dm^3 IAA. Cultures were kept

under 16 h light photoperiod at a temperature of 25 °C. Regenerating calli were defined as having, at least one shoot or root. The above described culture conditions were determined in experiments performed during 1986. The following factors were then tested: (1) explant size – in the range of 0.5–3 cm, (2) intact explants vs those divided into three segments, (3) supplementation of callus-inducing medium with different 2,4-D concentrations – 2 or 3.0 mg/dm^3, (4) supplementation of regeneration medium with 1.0 mg/dm^3 BAP +1.0 mg/dm^3 IAA or 1.0 mg/dm^3 2,4-D, (5) supplementation of rooting medium with 1.0 mg/dm^3 folic acid, (6) light/dark effect during the first 2 weeks of culture.

Differences between lines and crosses – F_1, F_2 and F_3 generations – were compared with a one-way analysis of variance (using Blisstransformation) and LSD and Schafee tests. One season of experiments was counted as one repetition (this concerns only lines). In the case of lines the results from each culture step have been described; in the case of crosses only those from hormone-free medium are reported. The broad-sense heritability of the number of regenerated shoots from shoot-regenerating explants and the percentage of finally-obtained plants (number of shoots/number of plants) were based on formulae given by Simmonds (1981).

Results

Characteristics of calli

The following types of calli were produced by explants: (1) primary callus – PC, white and friable, (2) weakly growing callus – WGC, white, thick nodular and soft watery, (3) intensively growing callus – IGC, white-yellowish and slightly nodular.

In-vitro response of ten inbred lines

The first differences between lines were observed in the third week of culture. They consisted of a different number of explants producing PC – from 10.48% in line L29 to 100% in lines Dw28, H363, L318, L299. During the next 2 weeks calli from some of the explants stopped growing whereas others continued to grow. Consistent differences between lines in the percentage of explants producing WGC and IGC were observed during this period of culture. Most of the explants producing IGC were in lines H363, D855 and Pw330 (97.86%, 94.02% and 92.86% respectively). None of the explants of lines L9, L29, L299 and H316 gave such a response. Some of the explants did not respond and they died after 3–4 weeks of culture. In lines L9 and L29 non-responsive explants were in a majority, as opposed to lines Dw28, H316, L318, D855 where no such explant was found. After subculture both explants produced WGC and IGC in hormone-free medium and only some of the explants previously forming IGC started to regenerate shoots or roots. Shoot regeneration was observed from pale yellow, slightly nodular IGC with green sectors and root regeneration from more compact, brownish-yellow IGC with cystalline islands.

Table 1. Results of immature inflorescences cultured in-vitro on hormone-free medium – lines

Line	Explant number	% of explants showing reaction:				
		1	2	3	4	5
Dw28	123	0.00	16.26	35.77	0.00	47.97
H363	140	0.00	2.14	5.00	16.43	76.43
L318	128	0.00	12.50	26.56	0.78	60.16
D855	117	0.00	5.98	38.46	1.71	53.85
H32	113	7.08	27.43	64.51	0.00	0.98
Pw330	84	7.14	0.00	10.71	25.00	57.15
L9	125	88.80	11.20	0.00	0.00	0.00
L29	124	89.52	19.48	0.00	0.00	0.00
L299	123	0.00	100.00	0.00	0.00	0.00
H316	96	1.04	0.00	0.00	98.96	0.00

1, NR; 2, WGC formation; 3, IGC formation; 4, IGC formation, root regeneration; 5, IGC formation, shoot regeneration

Table 2. Results of immature inflorescences cultured in-vitro on hormone-free medium – subsequent generations

Cross	Generation	Explant number	% of explants showing reaction:				
			1	2	3	4	5
Dw28 × H3	F_1	62	19.35	56.45	8.07	0.00	0.00
	F_2	264	0.00	57.20	42.42	0.38	0.00
	F_3	307	2.93	46.25	50.82	0.00	0.00
H363 × L31	F_1	59	0.00	94.92	5.09	0.00	0.00
	F_2	218	14.68	14.22	54.59	1.38	15.14
	F_3	278	2.52	60.07	32.73	4.68	0.00
D855 × L31	F_1	62	8.07	0.00	66.13	0.00	25.81
	F_2	300	32.00	43.00	2.33	12.67	10.00
	F_3	223	0.00	31.39	48.43	16.14	4.04
H32 × Pw3	F_1	65	32.31	21.54	46.15	0.00	0.00
	F_2	370	5.14	40.00	37.84	12.70	4.32
	F_3	301	3.66	93.02	1.99	1.33	0.00
L9 × L29	F_1	65	30.77	69.23	0.00	0.00	0.00
	F_2	390	24.62	75.13	0.00	0.00	0.26
	F_3	164	28.89	67.24	2.87	0.00	0.00
L299 × H31	F_1	75	4.00	81.33	0.00	0.00	14.67
	F_2	440	3.86	15.00	77.27	1.14	2.73
	F_3	244	0.00	41.39	44.67	12.71	1.23

No correlation was found between callus production and root and shoot regeneration in any line. The proportion of explants with a given response and the differences between lines remained unchanged from the 12th week on hormone-free medium (Table 1). Based on statistical analysis, the ten inbred rye lines were divided into six groups according to their homogeneity of response: (1) no response – L9 and L29, (2) producing a weakly growing, non-morphogenic callus – L299, (3) with an intensively growing, non-morphogenic callus – H32, (4) producing an intensively growing callus with regenerating roots – H316, (5) with an intensively growing callus and regenerating shoots and roots – H363 and Pw330,

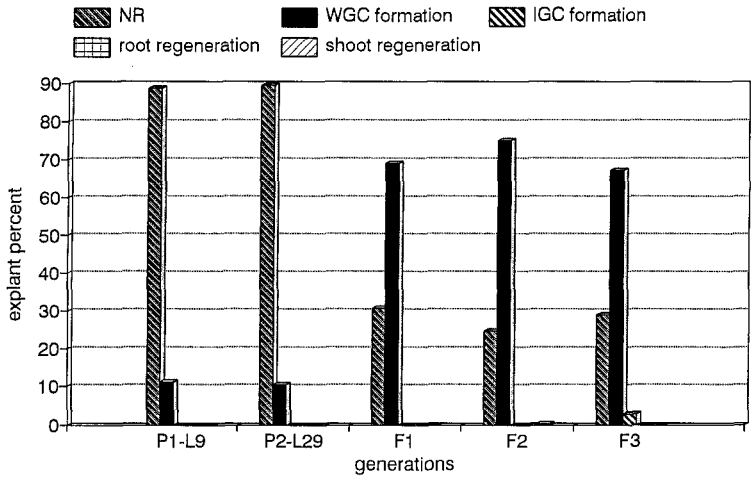

Fig. 1. The percentage of explants with different in-vitro responses in lines L9, L29 and their F_1, F_2 and F_3 progenies

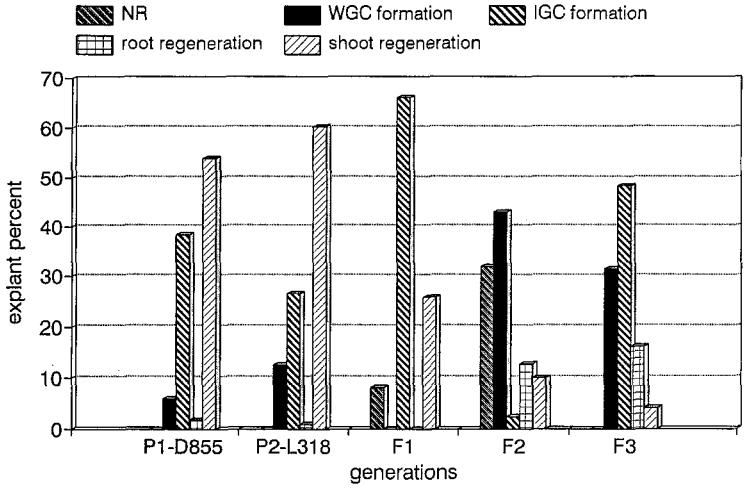

Fig. 2. The percentage of explants with different in-vitro responses in lines D855, L318 and their F_1, F_2 and F_3 progenies

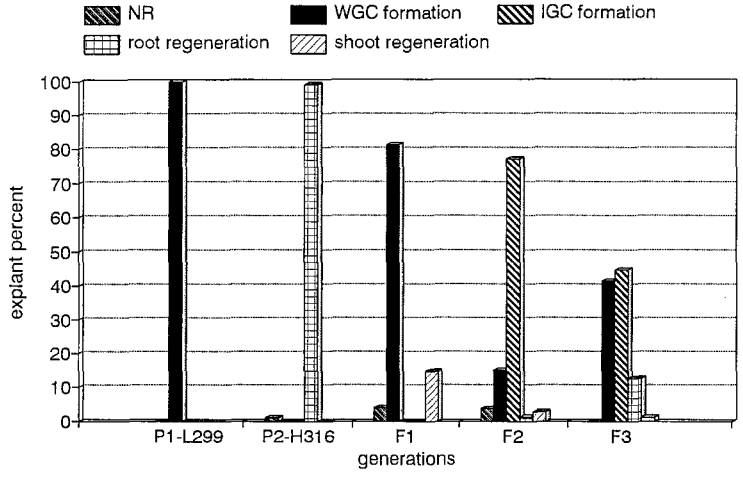

Fig. 3. The percentage of explants with different in-vitro responses in lines L299, H316 and their F_1, F_2 and F_3 progenies

(6) producing an intensively growing callus and regenerating shoots – Dw28, L318, D855.

No significant differences between the results from both seasons were found. Also no differences between lines belonging to the last two groups were evident in terms of the total number of plants obtained.

Analysis of crosses between lines with a different in-vitro response

In all generations of crosses between lines L9 and L29 belonging to the first group (non-responsive lines) most of the explants, about 70%, produced a weakly growing

callus and no more than 30% failed to react (Fig. 1, Table 2).

In crosses where one or both components originated from the sixth group, number H32 × Pw330, Dw28 × H363, D855 × L318 and L318 × H363, none or only a small of explants in the F_1, F_2 and F_3 generations regenerated shoots or roots, whereas the number of explants producing WGC increased in almost all cases (Fig. 2, Table 2). The cross between line L299 belonging to the second group (WGC formation) and line H316, defined as capable of root regeneration, resulted in nearly homogeneous F_1s where in excess of 80% of the explants gave the maternal response pattern. In the next generation most of the explants producing IGC, and in the F_3 the number of the explants with both types of response – WGC and IGC production – was nearly equal (Fig. 3, Table 2).

The broad-sense heritability of shoots and plant regeneration

The broad-sense heritability (hb^2) of shoot and plant regeneration was scored for lines H363, L318, D855, H32 and Pw330 because only in their progenies were shoots and plants obtained. Depending on the parental lines hb^2 reached 0.36–0.5 for the number of shoots regenerated from shoot-regenerating explants and 0.31 for the percentage of finally-obtained plants.

Discussion

Rye is known to be a poorly regenerable species. Untill now, relatively high and repetitive regeneration has been obtained from immature embryos (Rybczynski 1980) but regeneration is much lower from immature inflorescence (Eapen and Rao 1985; Krumbiegel-Schroeren et al. 1984). The low regenerative ability seems to be one of factors influencing the slow progress of biotechnological methods in this species in comparison with other cereals. The increasing importance of rye, however, emphasizes the need to obtain highly responsive genotypes.

The results presented in this paper prove the existence of genotypic differences in plant (root) regeneration ability and in-vitro callus formation. Five out of ten tested inbred lines in our experiment showed a relatively high regeneration ability, 47.97–76.43%, with explants regenerating shoots. Krumbiegel-Schroeren et al. (1984) also found genotypic differences in inflorescence cultures of rye. They obtained shoot regeneration from about 42% of explants in the case of the best-responding genotype ('1922') whereas in other genotypes only a few percent of explants produced an embryogenic callus and no plants were regenerated. Linacero and Vazquez (1990) also found that the genotype of inflorescence – donor plant was a very important factor implicated in the response in vitro. The differences concerned the number of responding explants, from 42.3–70.6%, and the percentage of regeneration (number of regenerating calli/number of embryogenic calli ×100), from 6.84–33.5%. These results confirm that a variability in the in-vitro response of immature inflorescences is to be expected in rye.

We did not find a correlation between IGC production and shoot regeneration. This is in agreement with already published results (Duncan et al. 1985) which indicated that there was no such correlation between callus production and plant regeneration from immature embryos in maize.

Most of the lines used in our experiment gave no homogeneous and repetitive response in spite of being highly homozygotic – inbred degree above S10. The exceptions were L9, L29, L299, H316, where the reaction was almost homogeneous. Investigations of esterase isoenzymes detected a lack of homogeneity only in the case of line Pw330, which consisted of a few sublines (Wochniak, personal communication). We suppose that the inhomogeneous response within the line could be caused not by genetic variability but rather by differences in the physiological status of the different size inflorescences (in range of 1–2 cm) of the explants. It is worth mentioning that mature plants of all the investigated lines had spikes of similar length. This means that the reaction variability was the same in all material. Krumbiegel-Schroeren et al. (1984) and Linacero and Vazquez (1990) found that callus induction and plant regeneration from immature inflorescences depended significantly on their size but involved much larger differences than in our experiments.

One of the aims of our study was to determine how the ability for regeneration from immature inflorescences is inherited in rye. Published work dealing with the genetical analysis of regeneration ability tends to follow one of three principal hypotheses: (1) the trait is controlled by single genes (one, two or three loci) that can cooperate in different ways and have a quantitative nature which is highly heritable (Reish and Bingham 1980; Charmet and Bernard 1984; Nadolska-Orczyk and Malepszy 1989), (2) regeneration ability is a polygenic trait and is strongly influenced by the environment (Tomes and Smith 1985), (3) it is a dominant trait and genes are designated usually as R or Rg (Reish and Bingham 1980; Komatsuda et al. 1989; Nadolska-Orczyk and Malepszy 1989). Dominant major and regulator genes, called TCR (tissue-culture response) and localized respectively on chromosomes 2D and 2B, were shown to be involved in the expression of the in-vitro response in wheat (Kaleikau et al. 1989b). Positive heterotic effects on androgenic plant regeneration in rye were emphasized by Flehinghaus et al. (1991).

A useful measure of regeneration ability is the heritability of some of its elements or steps (Nadolska-Orczyk

and Malepszy 1989). In our experiments the broad-sense heritability of shoot regeneration and of finally-obtained plants could be determined only for five of six lines classified as eligible for plant regeneration. Progenies obtained after crossing with line Dw28 did not regenerate shoots so this line could not be taken into account. The heritability was relatively low for both regenerated shoots and the percentage of finally-obtained plants (number of shoots/number of plants $\times 100\%$), respectively 0.36–0.5 and 0.31–0.67. This indicates a significant influence of non-heritable components for both these traits.

Results obtained in the present paper indicate that: (1) the ability for plant (or root) regeneration in rye is a recessive trait or, at least, that two dominant non-allelic complementary genes suppress it, (2) the heterotic status of the donor plants does not increase the regeneration ability, (3) the ability for callus formation is either a recessive trait or else that it too can be suppressed by a dominant gene(s), (4) the ability for producing WGC is controlled by complementary genes, (5) the production of both callus types can be regulated additionally by other genes, presumably involving dominant loci. Some similarities to the proposed model can be found in the work of Broda (1984). He showed that three recessive genes determined plant regeneration from hypocotyl and ovary-derived material in *Trifolium pratense*.

In conclusion, the in-vitro response of immature inflorescences in rye seems to be a trait controlled by a complicated polygenic system with different gene interactions, involving non-dominance of plant regeneration ability and a negative role for heterosis. Such mode of inheritance is rather rare both in di- and mono-cots.

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