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THE EFFECT OF PLANT GROWTH REGULATORS ON CALLUS INDUCTION AND REGENERATION OF HAPLOID PLANTLETS IN ANTHER CULTURE OF WHEAT

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Abstract: The experiment was conducted to assess the effect of different concentration combination of 2,4D, α-NAA and kinetin on callus induction and regeneration of haploid in anther culture of wheat. Potato-2 liquid medium supplemented with different concentration combinations was used for this purpose. Among all the formulations, efficient callus induction and plant regeneration were observed in liquid potato medium containing following concentrations (mg l^{-1}) of plant growth regulators: 2,4D-0.75; α-NAA-0.1 and kinetin 0.75. This formulation supported to produce haploid plantlets directly without transferring calli to regeneration medium.

Keywords: Wheat; Anther culture; Callus induction; Regeneration; Growth regulators

Introduction

Wheat is the leading food crop in world farming. It occupies over 30% of the crop area under all grain cultures (Bakumovsky, 1983). Improvement of wheat and creation of novel varieties are intimately related with the well being of people of the world. Today in order to create varieties of wheat by traditional plant breeding methods, 8-10 years of intensive work is required. The development of anther culture technology leading to haploid is a valuable tool in speeding up the crop improvement process, the application of which the time saving can be substantial to 3-4 years. But the success is restricted by two main factors; low frequency of callus induction and regeneration of plants. Medium composition, including organic and inorganic elements and growth regulators, genotype of the cultivars and culture environment after inoculation are the important factors of anther culture. The aim of this study was to optimize the medium composition, to increase the efficiency of callus induction and plant regeneration in wheat anther culture.

Materials and Methods

The research work was carried out in 1991 at the laboratory of cell Engineering, Lukyanenko Agricultural Research Institute, Krasnodar, Russia. The Russian spring wheat variety 'Drudzina' was taken as a donor plant for anther. The donor plants were grown in the field having plot size $3m^2$ and maintaining the density at the rate of 500 plants per square metre. The following growth regulators were tested in the potato-2 medium for callus induction: 2,4D, α-NAA and Kinetin. Young spikes still enclosed in leaf sheaths were collected in the morning hours (8 to 9 am) and were

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placed in a bucket with water and kept for 7 days in a cool room at a temperature of 3° to 5°C. After the completion of chill treatment, the spikelets were isolated and their sterilization was done with 0.1% solution of sodium hypochlorite for 15 minutes, followed by washing them with autoclaved distilled water under aseptic condition. Inoculation of anthers was done under laminar airflow with the help of forceps in petridishes containing 25ml liquid medium. Fifty anthers were inoculated in a dish. Then the cultures were kept in a dark room at 26°C for one month and after that the cultures were incubated in growth chamber maintaining temperature 22°C at day time and 18°C at night in a 16 hour daylight regime at about 10,000 lux. According to Table 1 and Table 2 a total of 27 different combinations of 2,4D, α-NAA and kinetin were used in this experiment for callus induction and to realize their subsequent effect on plant regeneration. Each combination consisted of 3 replications and 50 anthers were inoculated per replication. After 45 days of inoculation of anthers, calli were subcultured on Murashige and Skoog medium supplemented with 2, 4D – 0.25 mg l^{-1}, α-NAA- 0.25 mg l^{-1}, kinetin – 0.5 mg l^{-1}, sucrose – 3% and agar – 0.7% with pH – 5.8 for regeneration. Efficiency of callus induction and regeneration of plants were determined by the following formulae:

1. Frequency of callus induction (%)

$$= \frac{Number\ \ of\ \ anthers\ \ producing\ \ callus}{Number\ \ of\ \ inoculated\ \ anthers} x100$$

2. Frequency of plant regeneration (%)

$$= \frac{Number\ \ of\ \ regenerated\ \ plants}{Number\ \ of\ \ plated\ \ calli} x100$$

3. Frequency of green plant regeneration (%)

$$= \frac{Number\ \ of\ \ regenerated\ \ green\ \ plants}{Number\ \ of\ \ plated\ \ calli} x100$$

4. Frequency of albino plants regeneration(%)

$$= \frac{Number\ \ of\ \ regenerated\ \ albino\ \ plants}{Number\ \ of\ \ plated\ \ calli} x100$$

Results

Effects of different concentration combinations of considered factors on callus induction and plant regeneration are given in the Table 2. In the present study, calli were found to regenerate in each of the 27 combinations, which ranged from 2 to 41.9% of inoculated anthers. However, maximum callusing (41.9%) was obtained in 21st variant. Results of plant regeneration showed that regenerated plants occurred in 17 variants and among all the formulations, better results were observed in the 4th and 5th combinations and regeneration rate were 59.8% and 52.6% respectively from plated calli. Although most of the regenerated plants obtained from these combinations were albinos and frequency of callus induction was very low 7.8% and 6.3% respectively. Formulation of variant No. 21 has shown better performance in obtaining green regenerated plants, as well as callus responding. In this variant regenerated plants were 49.3% and out of these 42.9% were green and only 6.4% were albinos.

Table 1. Different concentration level of growth regulators (mg l^{-1}).

Growth regulators	Interval of variation	Concentration level of growth regulators		
		C_1	C_2	C_3
X_1 2,4D	0.25	0.25	0.50	0.75
X_2 α-NAA	0.15	0.10	0.25	0.40
X_3 Kinetin	0.25	0.25	0.50	0.75

Discussions

Butenko, Kholodov *et al.* (1972) reported that absence of phytohormones in the growth medium, cell division, its growth and differentiation do not take place. Among the phytohormones most important are auxin and cytokinin for wheat anther culture. In the present study 2, 4D and α-NAA as auxin and kinetin as cytokinin were used. Ouyang *et al.* (1973) found better callusing by increasing the concentration of 2,4D up to 25mg l^{-1}. At the same time Henry and Buyser (1981) and Zu (1978) observed that 2 mg l^{-1} of 2,4D is the most optimum concentration both for callusing and regeneration of plants. They also reported that with the increase in the concentration of 2, 4D from 1 mg l^{-1} up to 2 mg l^{-1} callus induction increased slightly but regeneration rate increased 2 to 3 times.

Table 2. Appearance of calli and regenerated plants in different concentration level of 2,4D, α-NAA and kinetin %.

Variants	Concentration level			% of callus from inoculated anthers	% of regenerated plants from plated calli		
	X_1	X_2	X_3		Total	Green	Albino
1	C_1	C_1	C_1	3.2	41.7	20.8	20.8
2	C_2	C_1	C_1	10.0	0.0	0.0	0.0
3	C_3	C_1	C_1	13.5	39.4	29.6	9.8
4	C_1	C_2	C_1	7.8	59.8	34.2	25.6
5	C_2	C_2	C_1	6.3	52.6	31.6	21.0
6	C_3	C_2	C_1	11.5	5.8	5.8	0.0
7	C_1	C_3	C_1	5.4	12.3	12.3	0.0
8	C_2	C_3	C_1	5.3	37.5	37.5	0.0
9	C_3	C_3	C_1	4.0	33.3	16.7	16.7
10	C_1	C_1	C_2	5.3	12.5	0.0	12.5
11	C_2	C_1	C_2	21.3	3.1	3.1	0.0
12	C_3	C_1	C_2	16.0	12.5	0.0	12.5
13	C_1	C_2	C_2	7.9	0.0	0.0	0.0
14	C_2	C_2	C_2	5.9	11.2	11.2	0.0
15	C_3	C_2	C_2	10.0	0.0	0.0	0.0
16	C_1	C_3	C_2	21.1	22.1	0.0	22.1
17	C_2	C_3	C_2	7.5	0.0	0.0	0.0
18	C_3	C_3	C_2	5.0	0.0	0.0	0.0
19	C_1	C_1	C_3	2.0	33.3	33.3	0.0
20	C_2	C_1	C_3	4.0	0.0	0.0	0.0
21	C_3	C_1	C_3	41.9	49.3	42.9	6.4
22	C_1	C_2	C_3	3.3	0.0	0.0	0.0
23	C_2	C_2	C_3	7.1	0.0	0.0	0.0
24	C_3	C_2	C_3	2.0	0.0	0.0	0.0
25	C_1	C_3	C_3	21.3	6.3	3.1	3.1
26	C_2	C_3	C_3	17.2	0.0	0.0	0.0
27	C_3	C_3	C_3	13.1	15.2	10.2	5.0

X_1 – 2,4D, X_2 - α -NAA and X_3 – Kinetin

In the present study, it was observed that 2,4D at the concentration of 0.75 mg l^{-1} with the combination of α -NAA and kinetin responded better for both callusing and regeneration of plants. George and Liang (1987) reported that concentration of α-NAA at 1.0 mg l^{-1} as the main phytohormone with the combination of 2,4D at 0.1 mg l^{-1} supported direct regeneration of plants in wheat anther culture. Similar trend was also observed in our experiment that medium containing α-NAA 0.1 mg l^{-1} with the combination of 2,4D - 0.75 mg l^{-1} and kinetin – 0.75 mg l^{-1} provided direct regeneration. This result was not in agreement with the findings of George and Liang (1987), who considered α-NAA as the main factor for direct regeneration. This dissimilarity may be due to use of different donor genotypes.

Huang (1985) reported that absence of kinetin in the growth medium sharply reduced regeneration of green plants. He also found that regeneration of green plants increased with the increase in concentration of kinetin. It was also observed in the present experiment that output of green regenerated plants reached 42.9% with increasing the concentration of kinetin up to 0.75 mg l^{-1}.

Conclusion

The present work has shown that the effects of combined use of two auxin, 2,4D and α-NAA and one cytokinin, kinetin at the concentration of 0.75 mg l^{-1}, 0.10 mg l^{-1} and 0.75 mg l^{-1} respectively were superior for both callus induction and regeneration of green plants in wheat anther culture, particularly for the variety 'Drudzina'.

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