Theor Appl Genet (1984) 68:375–379

Somatic embryogenesis and plant regeneration in callus from inflorescences of *Hordeum vulgare* × *Triticum aestivum* hybrids

C. C. Chu [1], C. S. Sun [1], X. Chen [2], W. X. Zhang [2] and Z. H. Du [2]

[1] Institute of Botany, Academia Sinica, Peking, China
[2] Institute of Crop Breeding and Cultivation, Chinese Academy of Agricultural Sciences, Peking, China

Received January 30, 1984; Accepted February 21, 1984
Communicated by Hu Han

Summary. Embryogenic callus cultures were obtained by culturing young inflorescence tissues of *Hordeum vulgare* cv. 'PF 51811' (2x) × *Triticum aestivum* cv. 'Chinese Spring' (6x) hybrids on 2,4-D-containing N_6 medium. After subculture for about 10 months the calli retained a high potentiality for somatic embryogenesis and plant regeneration. Of about 300 regenerated plants, approximately 100 were transplanted to potting soil. Eight embryoids and three regenerated plants examined had 28 chromosomes identical to the original hybrid plants, while one regenerated plant was found to be a mixploid composed of cells with 28 and 56 chromosomes. The possibility for obtaining amphiploid hybrids through tissue culture is discussed.

Key words: *Hordeum* × *Triticum*-hybrids – Somatic embryogenesis – Regeneration – Inflorescence – Chromosome doubling

Introduction

In spite of the difficulties of plant regeneration in species of *Gramineae,* remarkable progress has been made in this field in recent years. According to Vasil (1982 a, b), shoot differentiation from somatic calli has been achieved in more than 30 species of *Gramineae.* Among them, the regenerated plants of 10 species were produced through somatic embryogenesis. Recently, tissue culture techniques have been applied to propagate intergeneric hybrids and double their chromosomes (Nakamura et al. 1981). These techniques are also valuable in cell mutant selection and other genetic manipulation of cereals. This paper deals with somatic embryogenesis and plant regeneration in the intergeneric hybrid between *Hordeum vulgare* and *Triticum aestivum.* Chromosomal changes in the regenerated plants are also described here.

Materials and methods

Young inflorescences of *Hordeum vulgare* cv. 'PF 51811' × *Triticum aestivum* cv. 'Chinese Spring' and *H. vulgare* cv. 'PF 5801' × *T. aestivum* cv. 'Chinese Spring' hybrids were used for callus induction. These hybrids were produced in 1982 through in vitro embryo culture of the hybrid seeds. The inflorescences were surface sterilized with 70% ethanol for 1 min after which their outer leaves were removed. Each inflorescence was cut into 2–4 explants which were placed on the N_6 medium (Chu et al. 1975) supplemented with 2 mg/l 2,4-D for callus proliferation. After a 40 days induction period, the calli obtained were transferred onto the medium containing both NAA 0.1 mg/l and kinetin 1.0 mg/l for testing the ability of organ differentiation. The calli which exhibited shoot differentiation were further subcultured on the media containing 2,4-D 1.0 mg/l or both 2,4-D 1.0 mg/l and kinetin 1.0 mg/l. The effects of 2,4-D concentration on somatic embryogenesis and plant regeneration were tested. All cultures were maintained in a culture room at 28 °C under artificial light given by fluorescent tubes during the day. The regenerated plants were separated from calli and transferred to culture flasks containing N_6 basic medium for further growth. After the establishment of a more vigrous root system, the plants were transplanted into potting soil in autumn and later moved into the greenhouse.

For chromosome examination the young embryoids and shoot meristems of the regenerated plants were pretreated with 0.1% colchicine at 2–3 °C for 3 h and then fixed in 3:1 ethanol-acetic acid for 18 h. The fixed samples were treated with 1:1 HCl-ethanol for 10 min and then washed three times with 45% acetic acid. The macerated samples were squashed in modified Carbol fuchsine solution (Miller et al. 1971).

Results and discussion

Callus induction

After about one week of culture, white translucent calli proliferated from the cut areas of inflorescence axes

Table 1. Callus induction and plant regeneration from inflorescences of the *Hordeum vulgare* × *Triticum aestivum* hybrid

Hybrids	Length of inflorescences (cm)	No. of explants cultured	No. of callusing explants	No. of plant-producing calli
PF 51811 × 'Chinese Spring'	1.0–2.5	11	11	0
PF 51811 × 'Chinese Spring'	2.5–3.5	12	12	8
PF 51811 × 'Chinese Spring'	4.0	3	3	0
PF 51811 × 'Chinese Spring'	4.5–5.5	7	6	0
PF 5801 × 'Chinese Spring'	1.0–2.5	6	6	0
PF 5801 × 'Chinese Spring'	2.5–3.5	12	12	0

and each individual floscule in nearly every explant from all tested inflorescences (Fig. 1, Table 1). When the calli were transferred onto the differentiation medium containing 0.1 mg/l NAA and 1.0 mg/l kinetin, only those which derived from the 'PF 51811' × 'Chinese Spring' hybrid showed plant regeneration (Table 1). These totipotential calli were separated from the regenerated plants and subjected to subculture. The calli from another cross recombination did not develop any shoot buds, although roots and small green spots could sometimes be seen on their surface.

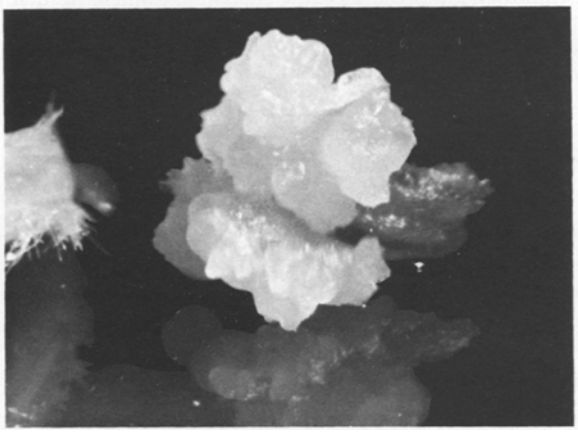

Fig. 1. Callus formation from explants of young inflorescences

Subculture of callus and somatic embryogenesis

On two subculture media the calli from the 'PF 51811' × 'Chinese Spring' hybrids gradually developed a kind of yellowish compact structure on their surface which were identified under the stereomicroscope to be embryoids. The young embryoids were globular or club-shaped (Fig. 2). Further development of the young embryoids followed different ways. A few of them underwent a zygotic embryogenic process and finally became mature embryoids with rather typical coleoptile, coleorzhiza and first leaf (Fig. 3). More often atypical embryoids of different kinds could be observed, including dish-shaped and cup-shaped embryoids (Fig. 4). In many cases, a large dish-shaped embryoid produced several curved leaves and subsequently developed multiple shoots (Fig. 5). This phenomenon contributed to the loss of apical dominance and the expansion of the organic zone of the embryoids caused by 2,4-D (Vasil 1982b). The frequency of embryoid formation on the medium with 1 mg/l 2,4-D was almost as same as on that with both 1 mg/l 2,4-D and 1 mg/l kinetin (Table 2). It means that kinetin is not necessary for somatic embryogenesis of the hybrid. The embryogenic callus cultures have been now subcultured for 10 months and still retain the ability for somatic embryogenesis at a high frequency.

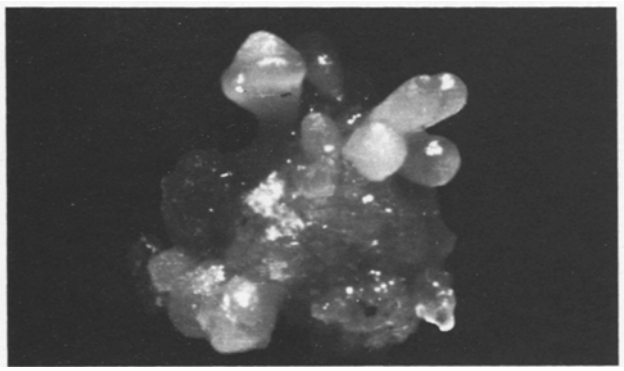

Fig. 2. Globular and club-shaped embryoids grown on surface of callus

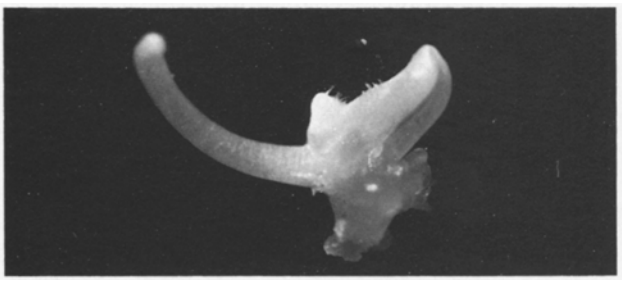

Fig. 3. A mature embryoid removed from callus, having coleoptile, coleorhiza and first leaf

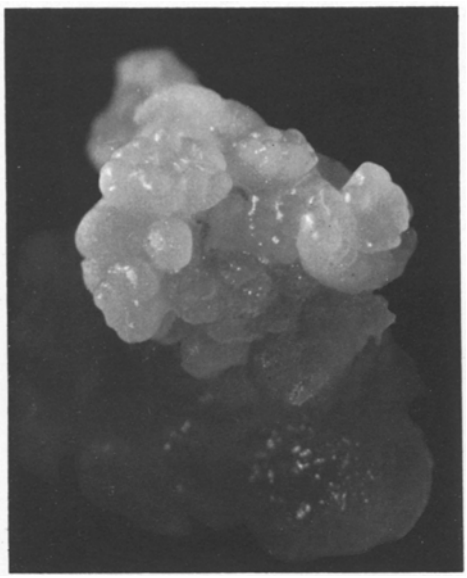

Fig. 4. Dish-shaped and cup-shaped embryoids in clusters on surface of callus

Fig. 5. Multiple shoots germinated from dish-shaped embryoid

Table 2. Embryoid formation from calli of *H. vulgare* cv. 'PF 51811' × *T. aestivum* cv. 'Chinese Spring' hybrid on subculture media

Hormones in media (mg/l)		No. of calli inoculated	No. of embryoid-producing calli	Frequency of embryoid formation (%)
2,4-D	Kinetin			
1.0	0	26	22	84.6
1.0	1.0	18	15	83.3

Factors affecting embryogenesis and plant regeneration

For testing the effects of 2,4-D on embryoid formation and plant regeneration, the friable calli without visible embryoids were transferred from the subculture medium to the media containing a different concentration of 2,4-D. During the 15–40 days of culture, embryoid formation was found on all tested media. Table 3 shows that the optimum 2,4-D concentration for embryogenesis was 0–1.0 mg/l and that when the concentration exceeded 2.0 mg/l, the frequency of embryogenesis sharply decreased. It should be mentioned that on 2,4-D-free medium the embryoids formed germinated rapidly into plantlets and there were no further callus proliferation and second round embryogenesis. On 2,4-D-containing media, however, new embryoids were continuously produced from the proliferating calli after the germination of first round embryoids.

As regards plant regeneration, the best results were obtained either on the media lacking 2,4-D, or that containing 0.1 mg/l 2,4-D (Table 3, Fig. 6). As mentioned earlier, the medium with 0.1 mg/l NAA and 1 mg/l kinetin was also suited to plant regeneration. Generally, 5–10 plantlets could be obtained from a single callus in 4–6 weeks. The plantlets regenerated on 2,4-D-free medium had well-developed root systems so they could be transplanted directly to soil. But on 2,4-D-containing media rooting of the plantlets was inhibited to varying degrees depending on the 2,4-D concentration used. In this case, before transplantation the plantlets should be transferred onto 2,4-D-free medium for root initiation and growth.

In addition to auxin concentration, a more essential factor for somatic embryogenesis was genotype of

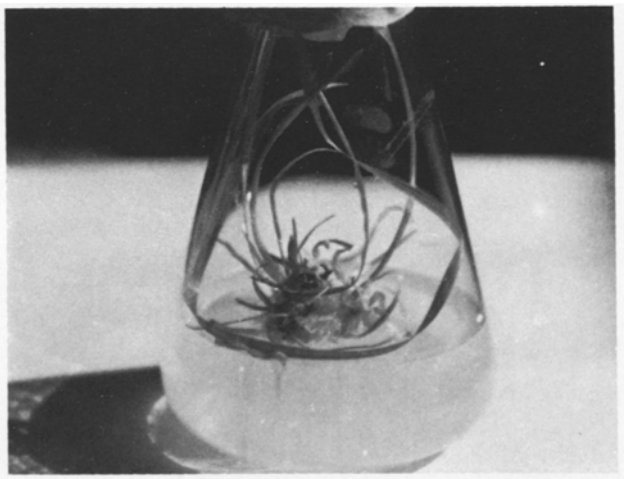

Fig. 6. Regenerated plants of *H. vulgare* cv. 'PF 51811' × *T. aestivum* cv. 'Chinese Spring' via somatic embryogenesis on 2,4-D-free medium

Table 3. The effects of 2,4-D concentration on embryoid formation and plant regeneration in calli of the *H. vulgare* cv. 'PF 51811' × *T. aestivum* cv. 'Chinese Spring' hybrid

2,4-D (mg/l)	No. of calli inoculated	No. of embryoid-producing calli	Frequency of embryoid formation (%)	No. of plant-producing calli	No. of plant regeneration (%)
0	25	22	88	21	84
0.1	25	23	92	20	80
0.5	25	19	76	13	52
1.0	25	18	72	13	52
2.0	25	12	48	8	32
4.0	25	10	40	5	20

donor plants. In this experiment, of two cross recombinations, only one showed totipotency. The developmental stage of the inflorescence at the time of excision was another critical factor. As shown in Table 1, the embryogenic calli only initiated from inflorescences 2.5–3.5 cm in length – this corresponds to the stages from floral formation to meiosis of PMC.

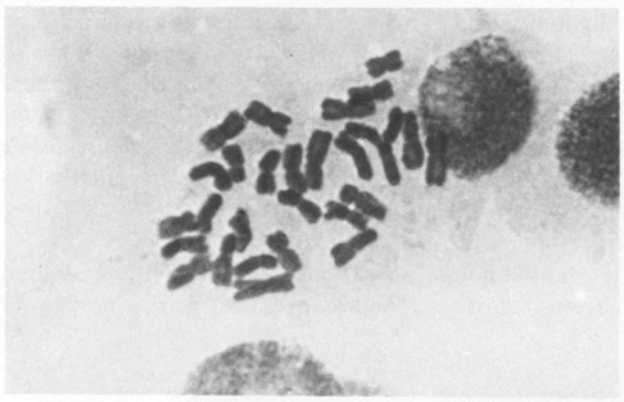

Fig. 7. Somatic metaphase of a regenerated plant (2n = 28)

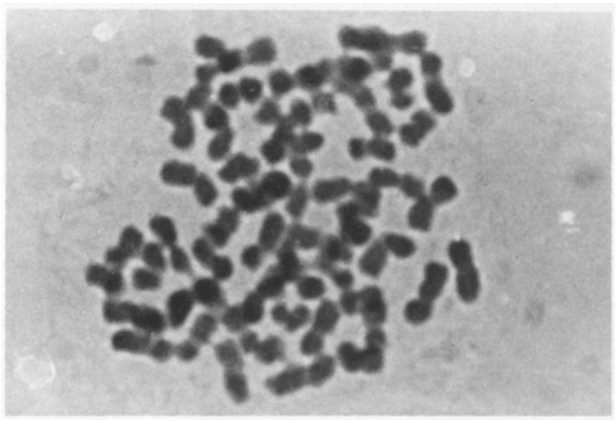

Fig. 8. Somatic metaphase of a mixploid regenerated plant, showing 56 chromosomes

Cytological examination

Cytological observation on the squashes with countable chromosomes revealed that eight examined young embryoids had a somatic chromosome number of 28-identical to the original hybrid plants. Of four examined regenerated plants, three were also dihaploid (2n = 28) (Fig. 7), but one plant was composed of cells with 28 and 56 chromosomes (Fig. 8). The existance of cells with 56 chromosomes implied that endoduplication or endomidosis occurred in the course of callus growth. In the literature on tissue culture, endoduplication and endomitosis in callus cultures were frequently mentioned (D'Amato 1977) and were successfully applied to obtain spontaneous diploids from haploid pollen plants (Nitsch et al. 1969; Hu 1978; Chu 1982). Partial success in doubling the chromosomes of a *Triticum crassum* × *Hordeum vulgare* intergeneric hybrid was also reported (Nakamura et al. 1981). Since all attempts aimed at obtaining amphiploids from the *Hordeum vulgare* × *Triticum aestivum* hybrid by colchicine treatment failed (Shepherd and Islam 1981), the procedures described here could be useful not only for propagating the hybrid but also for doubling chromosomes of the hybrid, of the approximately 300 regenerated plants obtained, about 100 have been transplanted into soil. Further cytological evaluation is in progress.

Acknowledgements. We wish to thank Prof. H. Hu for his critical reading of the manuscript and Mrs. Y. X. Wang for her technical assistance.

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