

Morphology and cytology of *Hordeum chilense* × *H. bulbosum* hybrids

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Summary. An interspecific hybrid between Hordeum chilense and H. bulbosum was produced. The hybrid resembles the male parent, but some characters from H. chilense are also present. Transgressive inheritance for other characters has also been observed. Neither chromosome instability nor homoeologous pairing was found.

Key words: Interspecific hybrid Hordeum chilense – Hordeum bulbosum – Chromosome elimination

Introduction

Interspecific hybridization is one of the steps in the study of phylogenetic relationships and in the process of interchange of genetic information between species.

Both Hordeum bulbosum and H. chilense are species that have been used in cereal breeding. When crossed with Triticum aestivum and T. turgidum cv. 'durum', H. chilense gives rise to stable hybrids and amphiploids (Martín and Cubero 1981), whereas H. bulbosum produces haploids when crossed with T. aestivum (Barclay 1975).

When *H. bulbosum* is crossed with other *Hordeum* species, both haploids and hybrids are produced (Kao and Kasha 1969; Lange 1971a, b; Rajhaty et al. 1964; Subrahmanyan 1978) with chromosome instability being a common feature.

In order to obtain material for further investigation on the elucidation of the nature of chromosome stability of hybrids involving *Hordeum* species, a hybrid between *H. chilense* and *H. bulbosum* was produced.

We present in this paper the morphology and chromosome behaviour of the hybrid.

Material and methods

H. chilense is a very polymorphic species which until very recently (von Bothmer et al. 1980) has not been extensively studied. We have followed the method of von Bothmer et al. (1980) to identify our *H. chilense* accessions.

Hordeum chilense (2n=2x=14) was obtained from Dr. Lange, Wageningen, The Netherlands, and Hordeum bulbosum (2n=2x=14) from Dr. Finch, Cambridge, UK. Both were vernalized at 4 °C with 6 h day-length during 2 months in a growth chamber. The plants were then transferred to a greenhouse in which, at the time of emasculation, the temperature and humidity range was 15-30 °C, and 40-95%, respectively. The methods of crossing, embryo culture and colchicine treatment were similar to the ones described by Jensen (1975).

For somatic chromosome counting, root tips of adult plants were treated with a saturated solution of bromonaphthalene, fixed on alcohol: acetic acid (3:1) and stained by the Feulgen procedure. For meiotic analysis, anthers were fixed and stained by the same procedure.

For nucleoli counting, a silver staining procedure (Fernández-Gomez et al. 1969) was used.

Results

From 30 pollinated florets of *H. chilense* with *H. bulbosum*, 9 embryos were obtained and 4 adult plants were established. Every plant was a hybrid with 14 chromosomes. We did not observe chromosome instability at any developmental stage. In somatic metaphase (Fig. 1) two satellited chromosomes from *H. chilense* were observed. When silver staining was applied, the results suggested (Table 1) that only the nucleoli from *H. chilense* were expressed.

The result of the study of meiotic chromosome pairing (Fig. 2) carried on pollen mother cells (PMC) is shown in Table 2.

The general aspect of the hybrid resembles H. bulbosum more than H. chilense and some characters show



Fig. 1. Mitotic metaphase of the hybrid *H. chilense* \times *H. bulbo-sum*. Nucleolar organizer *arrowed*



Fig. 2. Meiotic metaphase I of the hybrid H. chilense \times H. bulbosum (14 univalents)



Fig. 3. From left to right spikes of H. chilense, hybrid H. chilense \times H. bulbosum and H. bulbosum



Fig. 4. From left to right spikelets of H. chilense hybrid H. chilense \times H. bulbosum and H. bulbosum

Material	No. of cells examined	Mean	Range
Hordeum chilense	60	1.13	1-4
Hordeum bulbosum	60	1.38	1 - 2
H. chilense × H. bulbosum	60	1.46	1–2

Table 1. Number of nucleoli in somatic cells of H. chilense, H. bulbosum and the H. chilense \times H. bulbosum hybrid

Table 2. Average of association of chromosomes at metaphase I (MI) in the hybrid *H. chilense* \times *H. bulbosum* (range in brackets)

No. of PMC	Univalents	Bivalents 0.37 (0-2)	
80	13.25 (10–14)		

transgressive expression, for instance, vernalization requirements, stature, awn length or number of tillers. Spikelets and spikes aspect can be seen in Figs. 4 and 3.

The hybrid is completely sterile as was expected according to its meiotic behaviour.

Discussion

Subrahmanyan (1976) had reported on a hybrid *H. chilense* $(2x) \times H$. bulbosum (2x), but no details had been published except that 14 chromosomes were present. We found such a low chromosome pairing at metaphase I (Fig. 2 and Table 2) in our hybrid *H. chilense* \times *H. bulbosum* that homoeology between these species was almost non-existent. This implies, with some limitations, that phylogenetically both species are quite distant.

Thus, the behaviour of the 2 hybrids observed up to now suggests that chromosome elimination, common in hybrids involving *H. bulbosum*, is absent when *H. chilense* is used as a parent.

When trying to explain chromosome elimination, Bennett et al. (1976) suggested that the lack of expression of the nucleolar organisers of the eliminated genome could be the reason for elimination through a shortage of some particular proteins. However, our results show that only the nucleolar organizers of *H. chilense* are expressed in the hybrid *H. chilense* \times *H. bulbosum.* Therefore, in this case there is no direct relationship between the absence of expression of the nucleolar organizers and chromosome elimination in hybrids involving *H. bulbosum*. Thus, the hypothesis of Lange (1971b) could be the most likely explanation. Lange suggested that the quickening of the mitotic rhythm could be a possible cause of chromosomic disturbances in *H. vulgare* \times *H. bulbosum* hybrids. In this sense, both *H. bulbosum* and *H. chilense* are perennials, *H. vulgare* being annual. There have been no systematic studies on the relationships between life cycle-mitotic rhythm in cereals, but Nagl (1974), working with *Anthemideae*, pointed out that the mitotic cycle is shorter in annuals than in perennials. This hypothesis, however, remains to be confirmed.

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References

- Barclay IR (1975) High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256:410-411
- Bennett MD, Finch RA, Barclay IR (1976) The time rate and mechanism of chromosome elimination in *Hordeum* hybrids. Heredity 32:267-270
- Bothmer R von, Jacobsen N, Nicora E (1980) Revision of Hordeum sect. Anisolepis Nevski Bot Nots (Suppl) 133: 539-554
- Fernández-Gómez ME, Stockert JC, López Sáez JF, Gimenez-Martín G (1969) Staining plant cell nucleoli with AgNO₃ after formalin-hidroquinona fixation. Stain Tech 44:48–49
- Jensen CJ (1975) Barley monoploids and doubled monoploids. Techniques and experience. Barley Genet 3:316-345
- Kao KN, Kasha KJ (1969) Haploidy from interspecific crosses with tetraploid barley. Barley Genet 2:82–87
- Lange W (1971a) Crosses between *Hordeum vulgare* L. and *H. bulbosum* L. 1. Production, morphology and meiosis of hybrids, haploids and dihaploids. Euphytica 20: 14–29
- Lange W (1971 b) Crosses between Hordeum vulgare L. and H. bulbosum L. 2. Elimination of chromosomes in hybrid tissues. Euphytica 20: 181-194
- Martin A, Cubero JI (1981) The use of *Hordeum chilense* in cereal breeding. Cereal Res Commun 9:317–323
- Nagl W (1974) Mitotic cycle time in perennial and annual plants with various amounts of DNA and heterochromatin. Dev Biol 39:342-346
- Rajhathy T, Morrison JW, Symko S (1964) Interspecific and intergeneric hybrids in *Hordeum*. Barley Genet 1:195-213
- Subrahmanyan NC (1976) Interspecific hybridization, chromosome elimination and haploidy in *Hordeum*. Barley Genet Newsl 6:69-70
- Subrahmanyan NC (1978) Haploids and hybrids following interspecific crosses. Barley Genet Newsl 8:97-99