Identification of *Hordeum vulgare – H. bulbosum* recombinants using cytological and molecular methods

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Abstract

Barley (*Hordeum vulgare* L. subsp. *vulgare*) is an important crop and ranks fourth in overall production of the major cereal crops in the world. Like other cereal crops, barley suffers from a narrowing of its genetic base and susceptibility to diseases, pests and environmental stresses. *H. bulbosum* is a possible source of desirable genes for introgressing into barley to restore genetic diversity and improve current cultivars. Sexual hybridisation between barley and *H. bulbosum* is the main method for interspecific gene transfer in barley breeding but there are several barriers to overcome. Two of these are reduced recombination and the ability to identify recombinants quickly and efficiently. The aim in this thesis was to gain a better understanding of meiotic chromosomal behaviour in the two species and their hybrids and to improve the characterisation of recombinants from the hybrids.

To study the events during meiosis, synaptonemal complex (SC) analysis was carried out on the two species and two *H. vulgare* - *H. bulbosum* hybrids. The results indicated that there were interspecific and intraspecific variations in SC length. Mean SC length was positively correlated with recombination frequency but not related to genome size. This suggests that the ratios of mean SC length to genome size (SC/DNA) show divergence among these *Hordeum* examples. An hypothesis based on the conformation of chromatin associated with axial element, which is dependent on SC/DNA ratio, was presented to explain the relationship between SC length and recombination frequency.

Chromosome pairing in the two hybrids was determined by observation at pachytene and metaphase I (MI). Mean percentages of synapses were similar but there were different frequencies of MI pairing between these two hybrids, indicating that different mechanisms may regulate synapsis and MI pairing in the hybrids.

To investigate meiotic recombination, genomic *in situ* hybridisation (GISH) was performed on the two hybrids at MI and anaphase I (AI). It was observed that intergenomic pairing and recombination events occur in distal chromosome segments. A great discrepancy between mean pairing and recombination frequencies was observed in both hybrids and several possible reasons for this discrepancy were discussed. Hybrid 102C2 with high MI pairing had a significantly higher recombination frequency than the low pairing 103K5, suggesting that high MI pairing appears to be associated with high recombination in the hybrids. An interesting finding is that the ratio of recombination to MI pairing in 103K5 (1 : 8.9) is twice as high compared with 102C2 (1 : 17). However, the mechanism for this difference in the ratio between the two hybrids remains unknown.

Sequential fluorescence *in situ* hybridisation (FISH) and GISH were used successfully to localise the introgressions in selfed progeny from a tetraploid hybrid derived from chromosome-doubled 102C2 (102C2/colch). This procedure is fast, cheap and can efficiently detect and locate introgressions. Several disease-resistant recombinants were analysed in more details and leaf rust and powdery mildew resistance was associated with distal introgressions on chromosomes 2HS and 2HL (leaf rust) and 2HS (powdery mildew). It is possible that the leaf rust and powdery mildew resistances were closely linked in the distal region of 2HS. A considerable variation in introgression size was observed at similar chromosomal sites among the different recombinants, which will provide useful information for map-based cloning of genes.

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Abbreviations

µg	Microgram
µl	Microlitre
AFLP	Amplified fragment length polymorphism
AI	Anaphase I
AP	Alkaline phosphatase
bp	Base pair
CTAB	Cetyltriethylammonium bromide
CV	Coefficient of Variation
cv.	Cultivar
DAPI	4',6-diamidino-2-phenylindole
DIG	Digoxigenin
Fig.	Figure
FISH	Fluorescent *in situ* hybridisation
GISH	Genomic *in situ* hybridisation
HNPP	2-hydroxy-3-naphthoic acid-2'-phenylanilide phosphatase
kb	Kilobase
mg	Milligram
MI	Metaphase I
ml	Millilitre
nm	Nanometre
NOR	Nucleolar Organiser Region
PCR	Polymerase chain reaction
pg	Picogram
PI	Propidium iodide
PMC	Pollen Mother Cell

PSI	Pounds per square inch
PTA	Phosphotungstic acid
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNase	Ribonuclease
rpm	Revolutions per minute
SC	Synaptonemal complex
SC/DNA	Ratio of mean SC length to genome size
UP	Uranyl acetate and lead citrate
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume