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Survey of molecular diversity at *nud* locus in barley

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SUMMARY – Naked caryopsis trait in barley is controlled by a single recessive gene, *nud*, located on chromosome 1 (7H); it prevents adhesion of the lemma and palea to the caryopsis (hulless barley). To identify new markers closely linked to *nud,* to facilitate marker-assisted selection of the naked trait, we applied the RAPD protocol coupled with the Bulked Segregant Analysis strategy on the barley Proctor (hulled) x Nudinka (naked) mapping population. We identified two RAPD markers and only the most tightly linked, was successfully converted into a more specific SCAR marker. This together with other 6 markers were mapped onto a reference map. A naked/hulled barley germplasm was collected to individuate the haplotype structure surrounding the *nud* locus, employing most tightly linked markers. The response to leaf stripe disease was evaluated among a subset of the same germplasm, in order to investigate any possible associations between disease incidence and other recorded traits.

Introduction

Barley (*Hordeum vulgare*) hulless or naked caryopsis mutants are characterized by complete threshability because of lack of adherence of the two flowering glumelets to the kernel: for this reason they are preferred for food consumption. Most barley cultivars are hulled; nevertheless, in the last decade there was an increased interest for naked barley as a healthy food and as a new source of high-quality feed for swine (Bhatty, 1999). The naked trait in barley is controlled by a single recessive gene, called *nud*, located on chromosome 1 (7H) (Fedak *et al.,* 1972). A common problem of the breeding hulless barley varieties is the recognition of heterozygous from homozygous hulled plants. The use of molecular markers closely linked with the gene of interest could complement traditional phenotype testing to rapidly identify target genotypes in breeder's germplasm (Williams, 2003). Therefore, the first aim of this study was to find new PCR-based markers linked to the *nud* gene by means of Bulked Segregant Analysis (BSA), to facilitate marker-assisted selection (MAS) of the naked trait. Moreover, in order to evaluate the marker haplotype structure around the *nud* locus, we characterized a phenotypically differentiated barley germplasm (184 genotypes), including both hulled and naked forms, with one newly developed and other three SCAR markers, known to be closely linked to the naked gene (Kikuchi *et al*., 2003).

As a last objective, the same germplasm collection has been surveyed for resistance to leaf stripe (caused by *Pyrenophora graminea*), to investigate any possible associations between resistance to this seed-borne fungal disease, the naked seed phenotype and other morphological traits.

Refining the position of the *nud* locus

The application of the simple RAPD protocol coupled with the BSA strategy on the PN mapping population (derived from Proctor-hulled x Nudinka-naked cross), led to the identification of two RAPD markers (OP-D12 and OP-J14) closely linked to *nud*, that could be useful for naked barley breeding programs. In order to facilitate MAS of the naked trait, the most tightly linked RAPD OP-J14 was successfully converted into a specific SCAR marker (sJ14, that amplifies the "naked" allele). Given its linkage relationship with *nud* (0.9 cM), this newly developed marker with the sKT markers previously developed (Kikuchi, 2003) represent a useful tool for monitoring the naked trait, and could help breeders to discriminate rapidly heterozygous and hulless genotypes in early stages of the barley plant. In the PN reference map (Heun, 1991) the four most tightly linked flanking markers (sJ14, sKT2, sKT7, sKT3) are located within an interval of only 0.9 cM encompassing *nud* (Barabaschi *et al*., in press), and thus the precision of MAS for naked gene with any of these markers would be quite high. Borders of the region are given by the two SSRs (Bmag507 and Bmag120), that will allow comparisons with other maps.

Haplotypes in the region surrounding *nud* gene

The genetic variation and haplotype structure around the *nud* locus among 184 cultivated barley genotypes was investigated analysing the allelic variation at the four SCARs that showed the greatest association with the *nud* gene after linkage analysis. The haplotypic groups are indicated with numbers from 1 to 8 (Fig. 1). All the hulled genotypes (90) but one carried the haplotype 1 at the four loci (AAAA). Only one recombinant haplotype (BAAA; namely haplotype 2) was found. Among the naked genotypes, the most frequent haplotype was haplotype 3 (72%; 67 genotypes out of 93) with the B allele at the four loci examined. Further five recombinant variants were identified and validated: haplotypes 4, 5, 6, 7 and 8. Haplotype 4, ABAA, represented 17% of genotypes, while haplotypes 5, 6, 7 and 8 (in total about 11%) were rare recombinants. Genotyping at four marker loci has highlighted a greater haplotype variation in the 0.9 cM region for naked genotypes *vs* the hulled ones.

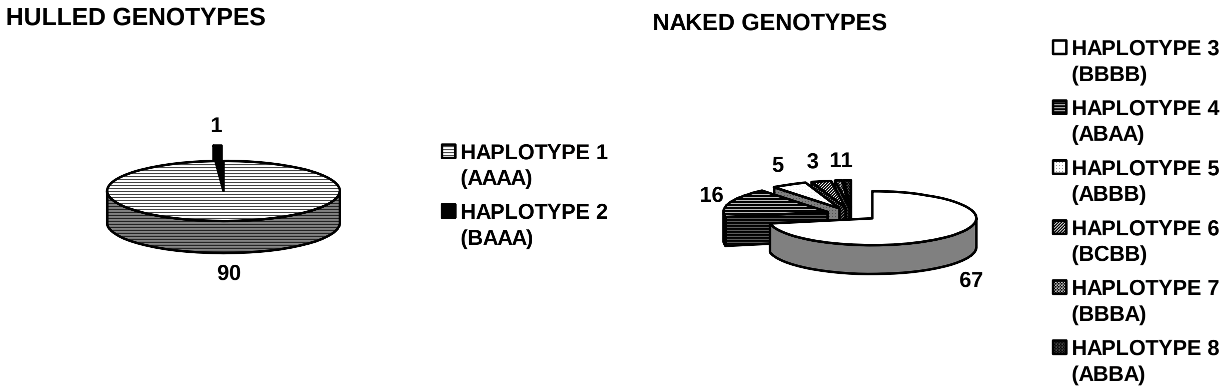

Fig. 1. Haplotypic groups frequencies.

Association between resistance to barley leaf stripe, naked phenotype and other traits

The resistance-susceptibility phenotype of 130 (naked/hulled) accessions of the same germplasm collection was verified by artificial inoculation with the highly virulent monoconidial isolate Dg2 of the pathogen *Pyrenophora graminea* in order to elucidate any possible associations between barley leaf stripe incidence and other morphological traits. The present study demonstrated that the naked phenotype is not associated to susceptibility to *Pyrenophora graminea* as suggested by Pecchioni et al., 1996. In fact the average level of infection was 39.15% for hulled genotypes and 41.45% for naked ones (Fig. 2a); the two groups did not differ significantly. The naked accessions have been further analysed separately, by considering association of the resistance trait with the most represented haplotypes (Fig. 2b). Genotypes with either haplotype 3 or 4 showed an incidence of the disease close to the average and comparable to that of the hulled genotypes, while a significantly higher resistance (15.55%) was observed for cultivars carrying the haplotype 5. These accessions were naked winter and facultative, mostly (3 out of 4) uzu-type dwarf and short-awned cultivars. After these preliminary observations, a correlation test was performed to investigate relationships between resistance to leaf stripe, plant height and awn length of naked barleys. The association was highly significant for both traits: leaf stripe resistant plants were significantly more frequent in naked dwarf cultivars than in normal ones (Fig. 2c) and also in short-awned cultivars than in medium- and in long-awned ones (Fig. 2d).

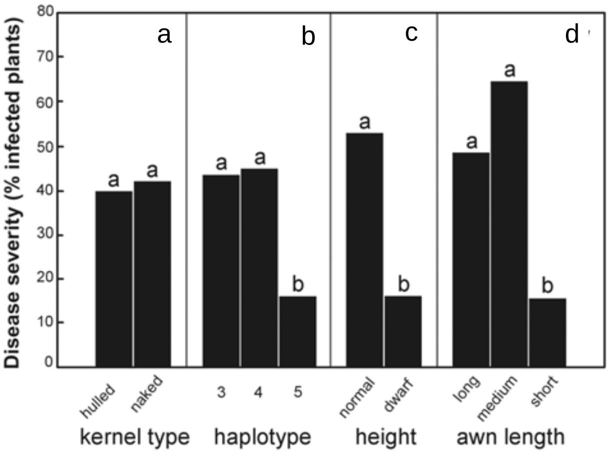

Fig. 2. Average leaf stripe severity (percentage of infected plants) in: (a) hulled vs. naked genotypes; (b) hulless haplotypic classes 3, 4, and 5; (c) hulless normal vs. dwarf genotypes; and (d) hulless accessions long-, medium- and short-awned. Within each trait, values with the same label are not significantly different for Dunn test at P=0.05.

References

Barabaschi, D., Francia, E., Campani, L., Toubia-Rahme, H., Stanca, A.M., Gianinetti, A. and Pecchioni, N. (in press). *Plant Breeding*.

Bhatty, R.S. (1999). *Cereal Chem*., 76: 589-599.

Fedak, G., Tsuchiya, T. and Helgason, S.B. (1972). *Can. J. Genet. Cytol*., 14: 949-957.

Heun, M., Kennedy, A.E., Anderson, J.A., Lapitan, N.L.V, Sorrels, M.E. and Tanksley, S.D (1991). *Genome,* 34: 437-447.

Kikuchi, S., Taketa, S., Ichii, M. and Kawasaki, S. (2003). *Theor. Appl. Genet*., 108: 73-78.

Pecchioni, N., Faccioli, P., Toubia-Rahme, H., Valé, G. and Terzi, V. (1996). *Theor. Appl. Genet*., 93: 97-101.

Williams, K.J. (2003). *Austr. J. Agricul. Res*., 54: 1065-1079.