Improving harvest index in oilseed rape (*Brassica napus*) through modifying canopy architecture

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Abstract

Oilseed rape (OSR) is in many ways more like a weed than a crop plant, partly because it has an indeterminate growth habit which produces many unproductive branches, flowers and pods. This results in wasted resources and poor canopy architecture. As a result harvest index (HI) in OSR is usually less than 25% which is very low when compared to other crop species such as wheat which has about 50%. Little, however, is known about how architectural traits function in determining the OSR canopy or how they are controlled genetically. Harvest index is difficult to study because it is the end product of many individual, component traits and is determined by the interactions of many genes and the variation resulting from the effects of the environment. We have tackled this problem by analysing many of the component traits involved in determining canopy structure, determining QTL for these traits and carrying out single marker analyses with candidate genes for traits likely to be significant in determining canopy structure identified within the model plant *Arabidopsis thaliana*.

We have used 188 lines of the TN doubled haploid population, a cross between the Chinese semi-winter variety Ningyou 7 and the European winter variety Tapidor, to study some of those traits thought to be significant in determining canopy structure. These include height, stem width, primary branch numbers and angles, pod numbers and angles, seed numbers per pod and seed size. Three field trials have been carried out in the UK in the 2005/06 season and the data collected are being used to develop a better understanding of how these individual traits combine to influence canopy architecture and HI, and also to identify QTL controlling them and whether selected candidate genes are associated with the observed trait variation.

The paper will present results obtained from this work characterising OSR canopy architecture and discuss its effects on HI and the genetic control of these traits and discuss how this might aid the identification of molecular markers for use in the development of more efficient sustainable crop production through the use of marker assisted selection for these traits.

Key words: Oilseed rape, *Brassica napus*, harvest index, canopy, architecture, QTL

Introduction

Oilseed rape (*B. napus*) is the most important oilseed crop grown in temperate agricultural regions but despite extensive breeding efforts, yields in the UK have not increased in recent years (DEFRA, 2006) and it remains an inefficient plant in several aspects of its growth. The indeterminate growth habit of this crop leads to the production of infertile or poorly productive branches with large numbers of unproductive flowers and pods. These characters, among others such as pod shattering before harvest, have led to oilseed rape being described as possessing 'many characteristics associated more with weeds than a crop plant' (Thompson and Hughes, 1986). This inefficiency is compounded by the fact that, in order to achieve high yields, it is necessary to have high input of N fertiliser which accounts for about 60% of the energy input into growing the crop (Kasterine and Batchelor, 1998). This makes it only marginally useful as a crop when grown for alternative uses such as bio-diesel production. This wasteful use of resources needs to be addressed to make this crop fit for the 21st century. An indication of the inefficiency of oilseed rape is seen in measurements of harvest index (HI; seed weight/seed + straw weight) where typical values in the UK are between 20 – 25% (compared to 50% and higher for wheat). HI is both physiologically and genetically complex and is determined by the actions of many individual traits each of which is likely to be quantitative and also environmentally sensitive.

The aim of the work described here is to break down HI into its smaller component traits and describe how these individually contribute to HI, how they interact together and also to study the genetic control of these traits through the identification of QTL. We aim to exploit the increasing amount of knowledge of developmental processes and gene function in the model crop *Arabidopsis* to identify potential candidate genes.

We report here on preliminary trials to establish the suitability of a doubled haploid (DH) population for the analysis of canopy architecture when grown under UK conditions. These trials were used to characterise the range of variation within the population for traits of interest and assess these for the presence of QTL, to test methodologies and to generate seed for a smaller representative subset of 94 lines prior to full scale trials in 2006-7.

Material and Methods

A doubled haploid population, TN; 188 individuals from a cross between the Chinese semi-winter variety Ningyou 7 and the European winter variety Tapidor was selected for this work (Qui et al, 2006). These parents show great contrast in many architectural traits, in addition to variation in a wide range of other traits of importance (IMSORB, 2002). We will report the results of two trials that were grown in the UK during the 2005-6 season. In September 2005, TN seed was drilled in a single randomized block at a typical seed density for UK conditions (*ca* 70 m^{-2}) in small plots (1.5 x 2 m) at Spalding, UK, while in the other trial at Newcastle, UK, TN seed were sown by hand in 4 randomised blocks consisting of 0.5m single rows containing 7 plants per row. Both trials were treated with fertilizer according to normal agricultural practice. The trial at Spalding was left unsprayed against diseases while at Newcastle Caramba was applied at 0.8 l/ha on 31st October 2005 to control light leaf spot and stem canker.

Plants were scored for establishment, general growth characters and disease susceptibility. The time at which the first flower per line opened was also recorded. When the plants reached maturity they were harvested for full analysis of traits in the laboratory. Three replicate plants per plot were harvested at Spalding while, at Newcastle, one plant per row was harvested from two of the blocks for the analysis of HI and one plant harvested from one block for the detailed analysis of individual traits expected to be linked with the determination of HI. For measurement of HI each plant was dried, the seed separated from the straw and weighed. The characters selected for detailed analysis included whole plant traits such as: height, length of terminal raceme, foot length (the distance from the lowest branch to the ground), number of nodes, fertile and infertile branches, basal stem width and flower and pod density. The fertile branched nodes and ten pods were selected from the terminal raceme and digitally recorded. From these images the angle of branches, pod and pedicel lengths and angles were measured using SigmaScan (SPSS). Seed number per pod and mean seed weight were measured from the ten pods.

Results

All lines of the TN population survived very cold spells during the winter, although segregation was observed for frost tolerance. Some lines flowered very early in January and were unable to set seed in the cold conditions. The population also showed segregation for susceptibility to phoma (stem canker) though there was a clear trial effect in that that the trial at Spalding was affected more severely than that at Newcastle. Lines which were affected showed severely reduced growth after flowering, however, traits established early in the season before flowering were unaffected.

Highly significant QTL were found for all these three traits with the increasing alleles for canker resistance, cold tolerance and later flowering coming, mostly, from the European parent Tapidor.

Data illustrating the range of variation within TN population for some traits of interest is given in figure 1 using data from Newcastle which was less affected by stem canker. Transgressive segregation was observed for all traits measured and showed a range of over 2.5 fold for the number of fertile branches to 1.1 fold for pod angle within the upper and lower 10% groups.

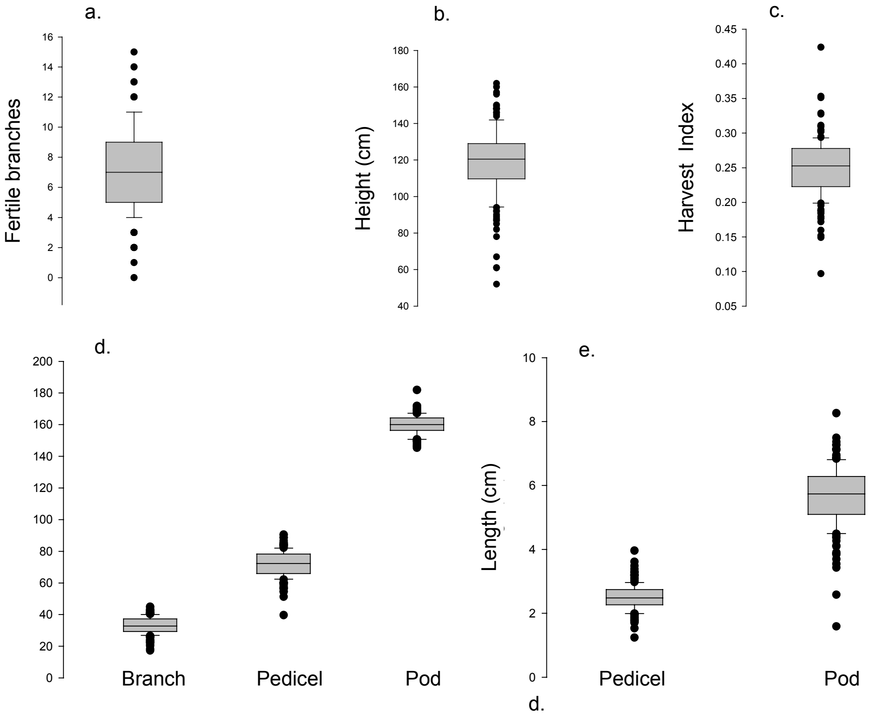

Fig. 1. Variation in traits associated with plant canopy architecture of the TN DH population, including a. number of fertile branches; b. height; c. Harvest Index; d. branch, pedicel and pod angles; e. pedicel and pod lengths.

QTL analysis of the data was carried out using both single marker analysis and interval mapping using MapQTL (Van Ooijen, 2004). Figure 2 shows examples of the QTL analysis carried out for both sites. Not surprisingly, a large number of QTL were found confirming that these traits are controlled by multiple alleles. Some QTL were found in both trials while some were specific to only one indicating that they are environmentally sensitive resulting in significant G x E effects. Despite the effect of site, several QTL were identified as being significant across different environments. These included pedicel length on N3, N9 and N16, pod angle on N2 and plant height on N3, N10 and N19.

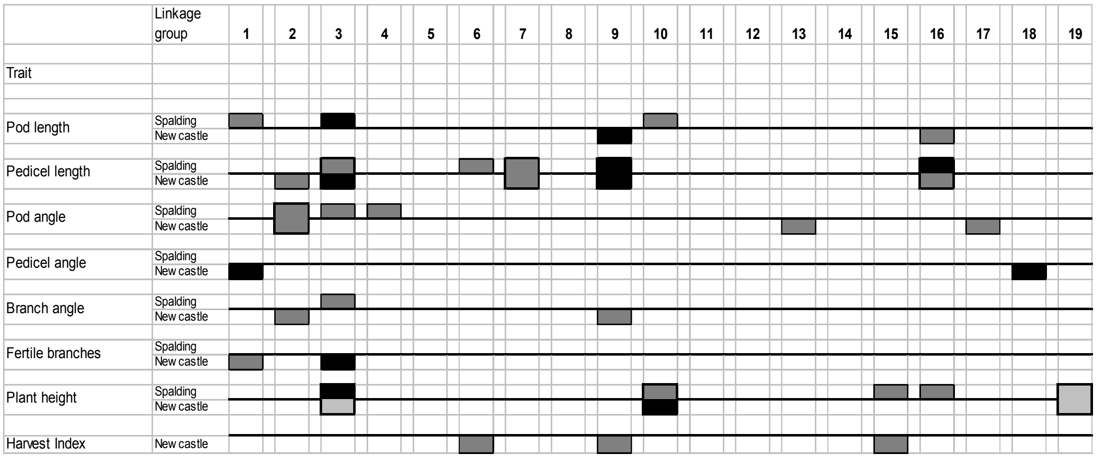

Fig. 2. Chromosomes with significant QTL for selected traits. LOD intervals > 4 ■; 3 – 4 ▨;2 – 3 ☐. Spalding data above line, Newcastle data below line.

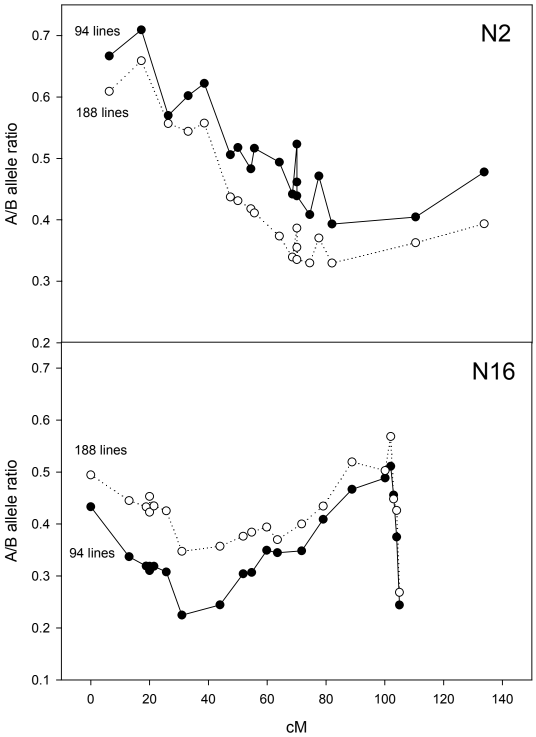

Figure 3. The effect of selection against canker susceptibility, frost sensitivity and early flowering on the allelic ratios of Tapidor (A) and Ningyou 7 (B) alleles on N2 & N16.

Flowering too early, frost sensitivity and canker susceptibility were seen as critical barriers to the successful use of the TN population in the UK. Those lines showing these characteristics were, therefore, preferentially removed to leave a set of 94 lines available for full yield trials in 2006-7. QTL analysis of these characters (data not given) showed that these traits were controlled by multiple loci with flowering too early, frost sensitivity and canker susceptibility mostly associated with alleles donated by the Chinese parent Ningyou 7. It was possible that removing these lines would potentially distort the allelic balance within the population and possibly reduce the extent of useful variation for traits of interest. However, a comparison of the means and distribution of these traits in the selected lines to those of the full population showed that they were essentially unaffected with the exception, of course, of three criteria used for the selection (data not given). The mean ratio of Tapidor to

Ningyou 7 alleles (A:B) ratio in the genome of the selected set of lines was compared to that of the full set.

Overall there was no consistent effect of selection though individual chromosomes, or parts of chromosomes, did show some alteration. Figure 3 illustrates the A:B ratio within the population for two chromosomes N2 and N16 where there were QTL for flowering time and which therefore might have been expected to be more affected by directional allelic selection. It can be seen that the allelic drift was toward increasing Tapidor alleles in N2 but towards Ningyou 7 alleles in N16. In both cases the allelic balance was mostly maintained between 30 – 70%.

Discussion

These data show that, when grown under UK conditions, the TN population exhibits a wide range of variation in traits which are likely to be significant in determining HI. These range from traits which determine the structure of the vegetative canopy as well as traits associated with the reproductive canopy. There are, however, two constraints on this population if it is to be useful under UK conditions. The first is the number of plants that may be successfully studied in the detail necessary to dissect a complex trait like HI into its component parts. Because these traits are very environmentally sensitive they are best studied in a range of environments. Studies seeking to determine both detailed analysis of a large number of traits and also determining the genetics of their control necessitate both sufficient replications within and between environments. The second is that the plants are agronomically suitable for the UK climate. A selection of 94 lines has therefore been made to allow sufficient replication for trait analysis while still allowing sufficient lines for QTL analysis. However, the detrimental effect of flowering too early when it was too cold for seed set, frost sensitivity and susceptibility to stem canker were all thought to arise from Ningyou 7 because Tapidor is a winter cultivar fully adapted to UK winter conditions and photoperiod. In contrast, Ningyou 7 is usually described as a ‘semi-winter’ cultivar which has a different photoperiod requirement for flower initiation, appears to require only a minimum vernalisation requirement and is unlikely to have been adapted to this level of cold. Additionally, Tapidor has been bred for resistance to stem canker in contrast to Ningyou 7 which was bred in China where stem canker is not endemic.

One approach to improving the productivity of oilseed rape is to improve the environment within the canopy for assimilation. Penetration of light is critical in the oilseed rape canopy and pods and leaves lower down the canopy are often working at sub-optimum levels. It is known, for instance, that canopies in which the pods are more vertical allow more light to penetrate (Fray, 1996) resulting in increased yields. The over-production of flowers and the extension of branches above the main canopy result in shading of the lower canopy so that optimising the shape, size and number of pods might be expected to firstly allow the more efficient use of resources and secondly to improve light penetration. In conclusion the results from these preliminary trials reported here indicate that the TN DH population contains within it sufficient variation to allow us to study the genetic control of individual traits related to canopy architecture and harvest index. The selected population of 94 lines will be better adapted to UK conditions although it will be necessary to reduce the effect of stem canker on the population. We have sown three fully replicated yield trials of these TN lines at Cambridge, Newmarket and Newcastle which will be fully analysed for traits associated with HI as described earlier.

Acknowledgements

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