New Brassica oilseed crops: '4B Brassica' crops for <u>B</u>iodiesel, <u>B</u>etter soils, <u>B</u>reak crops and <u>B</u>etter wheat

Julie A. Plummer
School of Plant Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia. Email: jplummer@plants.uwa.edu.au

ABSTRACT
The '4B Brassica' Crop program is a collaborative project between the University of Western Australia, the Department of Agriculture and Food Western Australia and key industry partners. This is a new approach to oilseed breeding in response to growing world demand for biodiesel. Our aim is to exploit for biodiesel production, the underutilized mustard species *Brassica juncea* (Indian mustard) and *B. carinata* (Ethiopian mustard) to develop a new '4B-Brassica' that combines the resilience of mustard species in high stress environments with the oil producing capacity of *B. napus*. '**4B** Brassica' crops for **B**iodiesel, **B**etter Soils, **B**reak Crop and **B**etter Wheat will provide an exciting new industry for regional Australia with low inputs and high oil yield under low rainfall conditions. Rapid breeding techniques will accelerate selection for drought tolerance, blackleg resistance, high seed yield and oil content. Seed meal can be recycled as fertilizer, and glycerine as a soil wetting agent. This provides a crop that better utilises available rainfall, transforms existing resource-based industries, improves soil quality, recycles carbon, reduces emissions, and addresses climate change.

Key words: Biodiesel, Breeding, Seed meal

INTRODUCTION

The cost of energy worldwide is increasing. In the last ten years, the price of crude oil has reached as high as $74/barrel and the long-term forecast is for further price increases as world oil reserves are progressively depleted. Australia relies on large-scale imports of fossil-derived diesel for industry and transport, which is unsustainable in the longer term. New sources of biodiesel will reduce Australia's reliance on diesel imports. Biodiesel is a renewable fuel that has similar properties to fossil diesel and emits less harmful combustion products. Biodiesel is produced by esterification of vegetable or animal oils. There are many potential sources of oil feedstock for biodiesel production, with plant-based oils being the most productive in terms of energy output / input ratios.

Canola (*Brassica napus*) dominates Australian oilseed production. In the peak year of 1999, 1.7 Mha of canola was sown, with Western Australia contributing the largest area at 830,000 ha. Historically, canola has been grown in areas with reliable rainfall and with a relatively high input of synthetic fertilisers. Canola oil and meal must also meet stringent quality requirements (low erucic acid, low glucosinolates) for food and feed uses. Alternative oilseed crops that are more resilient to lower rainfall and less fertile soils would be a good alternative to canola in many growing areas, especially marginal regions normally considered unsuitable for growing canola. Here oil would be for biodiesel and seed meal would be used as fertiliser. So our new approach is to develop 4B Brassica: Biodiesel, Better Soils, Break Crop and Better Wheat for marginal cropping areas.

4B BRASSICA PROJECT

The four components of the 4B Brassica Project: Biodiesel, Better Soils, Break Crop and Better are outlined below.

Biodiesel. Seed oil in *Brassica* species ranges from 35-44% (Downey and Rimmer 1993). Our research indicates seed oil concentration is inversely proportional to seed protein concentration (Si and Walton 2004; Gunasekera et al. 2006b) so a biodiesel *Brassica* would be bred for high oil yield per hectare rather than high protein. The impact of low rainfall and short seasons on protein concentration is greater than on oil yield (Si et al. 2003). Oilseed grain from a biodiesel *Brassica* would be processed for oil (preferably on farm or locally), and the meal (protein and fibre) would be recycled (e.g. back-freighted to the farm) to return N and organic matter to the soil and promote sustainability.

Oil quality in *Brassica* mustards vary greatly. Some mustard lines have high erucic acid concentrations conferring good lubricating qualities desirable in a fuel oil, but potentially a low melting point. The Australian Standards for Biodiesel include a minimum cetane level, oxidation stability and flashpoint temperature, and a required viscosity. Melting point, pour point and chemical stability will also be important considerations in breeding new *Brassica* oilseeds for biodiesel. Biodiesel properties will be examined with the assistance of our industry partners and using farm machinery and fleet vehicles.

There are many other uses of *Brassica* oil and glycerine, which will be developed as they become more available, and this will increase market potential. Currently the majority of polymers are derived from petroleum but certain products are based on vegetable oil and there is considerable scope for expansion. *Brassica* oils can be used as reactive agents in the manufacture of polyamides, polyesters, polyurethanes and nylon. Bioplastics derived from oilseed have good biodegradation properties. Pharmaceutical benefits are also being investigated for example, certain glucosinolates have cancer-preventative properties and consumption of *B. juncea* seed prevents insulin-resistance (Yadav et al. 2004).

Better soils – Biofertiliser. Mustard cake (meal) is a high quality organic fertiliser used extensively in India, Pakistan and areas of the Middle East. Mustard meal has a high C:N ratio and nutrients are released slowly as the meal is broken down providing a slow release fertiliser when applied at sowing (Balesh et al. 2005). Glycerine, a by-product of biodiesel production, is also used as a soil wetting agent. Use of mustard meals in fertilizer is new technology for Australia and methods for its use in our rainfed farming systems need to be developed; this will be accomplished in collaboration with our industry partners.

Break crop. Farmers have long observed the benefits of break crops, which can improve the yield of wheat. When wheat root diseases are present, yield of wheat after canola or Indian mustard can be 30% higher and protein 1.3% higher than wheat after wheat (Kirkegaard et al. 1997).

Better wheat. Wheat yields following mustards are higher due to reduced disease, greater nitrogen cycling and uptake by wheat, or improved water status. There are few viable rotational crops for wheat in low rainfall regions. Alternative oilseed crops with greater tolerance of marginal conditions will greatly benefit the wheat industry.

Our aim is to exploit the underutilised mustard species, *B. juncea* (AABB genome) and *B. carinata* (BBCC genome), to breed and select new oilseeds for biodiesel production. Breeders have introgressed genes from *B. juncea* and *B. carinata* to improve *B. napus* (canola) (Prakash and Chopra 1988; Schelfhout et al. 2004). This has been successful in reducing problems with late maturity, pod shattering, drought, and pest and disease susceptibility. However, despite these improvements, *B. napus* remains a crop that is best suited to areas of Australia with favourable growing conditions (i.e. high rainfall, high soil fertility achieved by synthetic fertilisers) where it is the most productive oilseed crop in terms of grain yield, oil content and oil quality (Mendham and Salisbury, 1995). A contributing factor to the lack of success of breeding canola for low-rainfall, low input agriculture has been the stringent food and feed requirements for canola that slow the rate of genetic improvement in other complex adaptive traits such as drought tolerance and nitrogen-use efficiency. Alternative *Brassica* oilseeds that already have these adaptive traits have historically been neglected because they do not meet the high quality oil and meal standards required of canola. However, with the surge in demand for biodiesel, which does not require the very high quality standards of canola, there is an opportunity to develop new, non-canola quality *Brassica* oilseed crops based on the mustards (*B. juncea* and *B. carinata*), which are well adapted to low rainfall, low input agriculture.

*B. juncea (*Indian mustard) usually outperforms canola when grown under dry conditions in Southern Australia, especially where water deficit develops after flowering (Lewis and Thurling 1994; Wright et al. 1995; Hocking et al. 1997; Gunasekera et al. 2006a). Indian mustard has higher yield than canola when sown late, such as in areas with short seasons or with a late break of the season. This is due to its greater ability to maintain leaves and produce dry matter after flowering under water deficit (Wright et al. 1995; Hocking et al. 1997). Our research has shown that across a range of genotypes of *B. napus* and *B. juncea* this is in part due to osmotic adjustment, which is positively associated with less yield loss and higher harvest index in plants grown under water deficit (Niknam et al. 2003). Osmotic adjustment is difficult to assess in the field but recently we have identified proline as a potential screening tool for *Brassica* (Ma et al. 2004). In addition, proline cosegregates with osmotic adjustment in interspecific crosses (Ma and Turner 2006). Populations segregating for proline concentration will be used to evaluate adaptation to drought complimented by experiments in low rainfall, short season environments. In *Brassica* oilseed crops, pod and seed development and hence yield, depend on photoassimilates from leaves and stored carbohydrates from vegetative organs (Pechan and Morgan 1985). Our research has shown that under low rainfall, high temperature and late sowing conditions *B. juncea* produces greater biomass than canola (Gunasekera et al. 2006a). Under water deficit *B. juncea* has less dependence on redistribution of stored carbohydrate and a greater access to current assimilate during pod filling than canola (Hocking et al. 1997). Water use efficiency of *B. juncea* is higher than canola (Angus et al. 1991). Our research shows these factors provide greater tolerance to stressful environments in *B. juncea*, indicated by greater phenotypic stability, compared with canola. However *B. juncea* has a relatively low harvest index compared with canola and this could be improved by breeding and selection (Guneshekara et al. 2006a, b; Ma and Turner 2006).

B. carinata (Ethiopian mustard) has the breeding advantages of deep rooting and high tolerance to heat, drought and saline conditions (Kumar et al. 1984; Malik 1990). It has large seeds with high erucic acid, high glucosinolate and high protein content. There is substantial variation in flowering time and oil content among Ethiopian germplasm including early maturing and high oil yielding lines (up to 42%) (Rakow and Getinet 1998).

Both *B. juncea* and *B. carinata* have other advantages including enhanced seedling vigour and a broad basal branching habit which are important for fast root and leaf development enabling better use of light, capture of soil water, reduced evaporation from the soil surface and better competition against weeds. Their resistance to pod shattering (Prakash and Chopra 1988; Rakow and Getinet 1998) is highly desirable as swathing is not required and harvesting is simplified, compared with canola.

B. napus (canola) is host to a number of fungal pathogens but Blackleg, caused by *Phoma lingam*/*Leptosphaeria maculans*, is the most important disease of *Brassica* oilseed crops in Australia and worldwide (Plieske and Struss 2001). The *Brassica* B genome is an important source of resistance. Canola breeding lines with high resistance to *L. maculans* races have been selected from interspecific crosses between *B. juncea* and *B. napus* both at the seedling (cotyledon) and adult stages (Chèvre et al. 1997; Saal et al. 2004). Variation among the lines suggests that several genes may be effective on their own and thus it may be possible to combine genes providing a more robust resistance. This could be achieved with the assistance of molecular markers that are linked to specific sources of blackleg disease resistance genes (Saal et al. 2004; Saal and Struss 2005). Research on resistance to Australian *L. maculans* isolates indicates a higher level and more widespread resistance within *B. juncea* than resistant *B. napus* lines (Purwantara et al. 1998). Further, *B. carinata* lines generally have high levels of resistance to blackleg and are more resistant than *B. juncea* lines (Gugel et al. 1990). Thus lines derived from hybridisation between *B. juncea* and *B. carinata* should have a high level of resistance. This will be examined in our well established disease nursery for blackleg.

Resistance to other diseases and pests exists within these alternate *Brassica* species. *B. juncea* is resistant to black rot (*Xanthomonas campestris*) and *B. carinata* is resistant to insect pests such as flea beetles and has high levels of resistance to *Alternaria* leaf spot and powdery mildew (Tonguc and Griffiths 2004; Westman et al. 1998).

There is a wealth of *Brassica* genetic material available to this project. We have access to a total of 2500 accessions of *B. juncea* and *B. carinata* from the Vavilov Institute (St Petersburg, Russia) and the Australian Temperate Field Crops collection (Victoria). Along with these natural collections, we will access interspecific progeny developed in other projects, such as *B. juncea*-like progeny from *B. napus* x *B. juncea* crosses and *B. carinata*-like progeny from *B. napus* x *B. carinata*. These genetic materials have only been partly screened as projects concentrated primarily on *B. napus*-like progeny. We have also negotiated research agreements with Prof. Jinling Meng (Huazhong Agricultural University, Wuhan, China) and Assoc. Prof. Phillip Griffiths (Cornell University, USA) to access a range of interspecific *Brassica* material. This project will proceed on several levels. Currently available materials will be screened and selected for 4B Brassica crops, selected materials will be further bred and selected and new materials will be developed for future oilseed breeding. This will provide a supply of improving lines for commercial production of new oilseed crops. These new 4B Brassica oilseeds will provide an important alternative feedstock for the developing biodiesel industry, especially in regional areas.

ACKNOWLEDGMENTS

I would like to acknowledge my colleagues and partners in this project from the University of Western Australia (Guijun Yan, Wallace Cowling, David Turner, Matthew Nelson, Margaret Campbell), the Department of Agriculture and Food Western Australia (Mohammad Amjad and Graham Walton) and support from members of the following companies: Aztech, Richgro, Western Biopower, Custom Composts, and farmer groups: WANTFA, Liebe Group, Facey Group and Holt Rock Group. I also appreciate advice provided by Clive Francis and Kadambot Siddique.

REFERENCES

Angus, J.F., A.F. van Herwaarden and G.N. Howe, 1991: Productivity and break crop effects of winter-growing oilseeds. Aust. J. Exp. Agric. 31, 660-677.

Balesh T, F. Zapata and J.B. Aune, 2005: Evaluation of mustard meal as organic fertilizer on tef (*Eragrostis tef* (Zucc) Trotter) under field and greenhouse conditions. Nutrient Cycling Agroecosys. 73, 49-57.

Chèvre, A.M., P. Barret, F. Eber, P. Dupuy, H. Brun, X. Tanguy, and M. Renard, 1997: Selection of stable *B. napus-B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans).* 1. Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor. Appl. Genet. 95, 1104-1111.

Downey R. K. and S. R. Rimmer, 1993: Agronomic improvements in oilseed Brassicas. Adv. Agron. 50, 1-66.

Gugel R.K., G. Seguin-Swartz and A. Petrie, 1990: Pathogenicity of three isolates of *Leptosphaeria maculans* on *Brassica* species and other crucifers. Can. J. Plant Pathol. 12, 75-82.

Gunasekera C.P., L.D. Martin, K.H.M. Siddique KHM, and G.H. Walton, 2006a: Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*B. napus* L.) in Mediterranean-type environments I. Crop growth and seed yield. Eur. J. Agron. 25, 1-12.

Gunasekera C.P., L.D. Martin, K.H.M. Siddique, and G. H. Walton, 2006b: Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*B. napus* L.) in Mediterranean-type environments II. Oil and protein concentrations in seed. Eur. J. Agron. 25, 13-21.

Hocking P.J., J.A. Kirkegaard, J.F. Angus, A.H. Gibson, E.A. Koetz, 1997: Comparison of canola, Indian mustard and Linola in two contrasting environments. I. Effects of nitrogen fertilizer on dry-matter production, seed yield and seed quality. Field Crops Res. 49,107-125.

Kirkegaard J.A., P.J. Hocking, J.F. Angus, G.N. Howe, and P.A. Gardner, 1997: Comparison of canola, Indian mustard and Linola in two contrasting environments. II. Break-crop and nitrogen effects on subsequent wheat crops. Field Crops Res. 52, 179-191.

Kumar A., P. Singh, D.P. Singh, H. Singh, and H.C. Sharma, 1984: Differences in osmoregulation in *Brassica* species. Ann. Bot. 54, 537-541.

Lewis G.L. and N. Thurling, 1994: Growth, development, and yield of three oilseed *Brassica* species in a water-limited environment. Aust. J. Exp. Agric. 34, 93-103.

Niknam S.R., Q. Ma, and D.W. Turner, 2003: Osmotic adjustment and seed yield of *Brassica napus* and *B. juncea* genotypes in a water-limited environment in south-western Australia. Aust. J. Exp. Agric. 43, 1127-1135.

Ma Q. and D.W. Turner, 2006: Osmotic adjustment segregates, and is positively related to seed yield, in F3 lines of crosses between *Brassica napus* and *B. juncea* subjected to water deficit. Aust. J. Exp. Agric. 46, 1621-1627.

Ma Q., D.W. Turner, D. Levy, and W.A. Cowling, 2004: Solute accumulation and osmotic adjustment in leaves of *Brassica* oilseeds in response to soil water deficit. Aust. J. Agric. Res. 55, 939-945.

Malik R.S, 1990: Prospects for *Brassica carinata* as an oilseed crop in India. Exp, Agric, 26, 125-129.

Mendham N.J. and P.A. Salisbury, 1995: Physiology: crop development, growth and yield. Brassica oilseeds. CABI Publishing, Wallingford

Pechan P.A. and D.G. Morgan, 1985: Defoliation and its effects on pod and seed development in oil seed rape (*Brassica napus* L.) J. Exp. Bot. 36, 485-468.

Plieske J. and D. Struss, 2001: STS markers linked to *Phoma* resistance genes of the *Brassica* B-genome revealed sequence homology between *Brassica nigra* and *Brassica napus*. Theor. Appl. Genet. 102, 483-488.

Prakash S. and I. Chopra, 1988: Introgression of resistance to shattering in *Brassica napus* from *B. juncea* through non-homologous recombination. Plant Breed. 101, 167-168.

Purwantara A., P.A. Salisbury, W.A. Burton, B.J. Howlett, 1998: Reaction of *Brasica juncea* (Indian mustard) lines to Australian isolates of *Leptosphaeria maculans* under glasshouse and field conditions. Eur. J. Plant Path. 104,895-902.

Rakow G. and A. Getinet, 1998: *Brassica carinata* an oilseed crop for Canada. Acta Hort. 459, 419-426.

Saal B., H. Brun, I. Glais, and D. Struss, 2004: Identification of a *Brassica juncea* derived recessive gene conferring resistance to *Leptosphaeria maculans* in oilseed rape. Plant Breed. 123, 505-511.

Saal B. and D. Struss, 2005: RGA-and RAPD-derived SCAR markers for a *Brassica* B-genome introgression conferring resistance to blackleg in oilseed rape. Theor. Appl. Genet. 111, 281-290.

Schelfhout C.J., R. Snowdon, W.A. Cowling, and J.M. Wroth, 2004: A PCR based B-genome-specific marker in *Brassica* species. Theor. Appl. Genet. 109, 917-921.

Si P., R.J. Mailer, N. Galwey, and D.W. Turner, 2003: Influence of genotype and environment on oil and protein concentrations of canola (*Brassica napus* L.) grown across southern Australia. Aust. J. Agric. Res. 54, 307-407.

Si P. and G.H. Walton, 2004: Determinants of oil concentration and seed yield in canola and Indian mustard in the lower rainfall areas of Western Australia. Aust. J. Agric. Res. 55, 367-377.

Tonguc M. and P.D. Griffiths, 2004: Transfer of powdery mildew resistance from *Brassica carinata* to *Brassica oleracea* through embryo rescue. Plant Breed. 123:587-589.

Wright P.R., J.M. Morgan, R.S. Jessop, and A. Cass, 1995: Comparative adaptation of canola (*Brassica napus*) and Indian mustard (*B. juncea*) to soil water deficits: yield and yield components. Field Crops Res. 42, 1-13.

Westman A.L., S. Kresovich, and M.H. Dickson, 1998: Regional variation in *Brassica nigra* and other weedy crucifers for disease reaction to *Alternaria brassiciola* and *Xanthomonas campestris* pv. *campestris*. Euphytica 106, 253-259.

Yadav S.P., V. Vats, A.C. Ammini, and J.K. Grover, 2004: *Brassica juncea* significantly prevented the development of insulin resistance in rats fed fructose-enriched diet. J. Ethnopharm. 93, 113-116.