ASSISTING NEW WHITE LUPIN PROCESSING INDUSTRIES BY BREEDING LARGER- AND SMALLER-SEEDED CULTIVARS

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Abstract

Two cultivars of white lupin with smaller (Minibean) or larger (Maxi) than normal seeds have been developed in a 25-year program of crossing, selection, induced mutagenesis and field testing. Over 90% of the seeds of Minibean are less than 10 mm in minimum diameter whereas 55% of the seeds of Maxi are larger than 10 mm in diameter. Both cultivars flower in mid-season, but mature late, especially Maxi, which requires a cool, moist late spring and early summer to maximise seed size. Maxi produces large seeds which attract a premium as food in Middle Eastern countries. The smaller seeds of Minibean are better suited to sowing and harvesting with standard cereal machinery, and give higher plant densities, and hence higher yield potential, at standard sowing rates. The grain is aimed at on-farm, feed-lot finishing of stock, and as split grains and flour in various foods and feeds.

Key words: Lupinus albus, white lupin, large-seeded, small-seeded, new cultivars, food.

The Mediterranean white lupin has been cultivated in parts of WA, NSW, and Victoria since the release of the late-maturing cultivar, Hamburg, and the early maturing cultivars Ultra and Kiev Mutant (1, 2, 3), to provide, initially, a high-protein stock feed supplement (5), and, more recently, a new source of a traditional, nutritious food for Middle Eastern people. The latter market pays a premium for larger seeds, especially those with a minimum diameter greater than 12 mm, but large seeds are easily damaged by cereal sowing and harvesting machinery. A smaller seed size would be advantageous for on-farm feed-lotting to finish lambs and cattle, because seed damage would be reduced. Also, crop density and yield potential would be increased at the standard sowing rate. Less damage and loss would occur during de-hulling to produce high protein food and feed ingredients, *eg*. by M. C. Croker Pty Ltd, Cootamundra.

A crossing and selection program was initiated by CSIRO in 1972. Although the genetic variability in F_3 families suggested that cultivars with higher yield (6) should be attainable through selection, this was prevented by high genotype x environment interaction in later years and other sites (4; R. N. Oram unpub.) Mutagenic treatments were then applied to increase the variability in several traits. This paper describes the origin and characteristics of two new cultivars arising from this combined crossing, mutagenesis, and selection program. One cultivar with thicker, broad seeds may assist the whole seed export trade, and the other with small seeds may assist manufacturers of food and feed ingredients.

Materials and methods

Crosses among eight low alkaloid and six high alkaloid parents gave 232 low alkaloid F_3 families in 38 F_2 groups (6). F_2- and F_3-derived families were yield-tested at Wagga Wagga and Canberra in 1975-1977. 163 single F_4 plants were selected from 13 crosses which had performed well at both sites as bulks during 1975. This "CW" population was re-selected at Canberra for uniformity of mid-season maturity, blue flower colour and low alkaloid content. To create additional variability, seeds were mutagenised with ^{60}Co?<F"Symbol"g-rays at doses of 200 to 400 Gray, or with 0.1, 0.3 or 0.5% *iso*-propyl methanesulfonate or ethyl methanesulfonate in pH 7.0 phosphate buffer for 8 h. Single plant selection was practised in 2-row plots at Canberra and Wagga Wagga in the M_2, M_3 and M_4 generations, after which three related large-seeded families were bulked. This bulk and one small-seeded family were re-selected for the respective seed sizes and for agronomic traits in 1990-1992. Pure seed of the two stable lines was produced and tested in NSW Agriculture core trials in 1994-1996. The alkaloid contents of seed

samples of both lines grown at Wagga Wagga in 1992-1994 and in S.A. in 1994 were assayed by the W.A. State Chemistry Laboratory.

In 10 lines grown at Wagga Wagga and Cowra in 1995, seed size distributions were determined by shaking 1-3 kg samples through a nest of sieves with circular holes of 8, 10, 11, 12 mm diameter. The percentage by weight of these fractions were transformed to arc sin?<F"WP IconicSymbolsA"**/**percentage before analysing the variance in each fraction, using the line x site interaction as the error term. Also, 50 seeds of Maxi and breeding line A75Bo9-2-3 from each site were weighed individually.

To further characterise Minibean and Maxi, two or three plants of each line, and of six Australian and overseas cultivars, were grown in 25 cm white pots in a minimally heated glasshouse in 1996. Seeds were inoculated with group G rhizobia and sown in late April in sand-vermiculite. The two pots of each line were arranged in a two-replicate randomised block design. The pots were watered twice-weekly with a half-strength Hoagland's nutrient solution, but this caused leaf chlorosis, apparently due to a mineral imbalance. Growth returned to normal after changing to a nitrogen-free, modified Hoagland's solution. Few pods were set on the primary inflorescences, but the two later orders of flowers set pods normally; these were harvested separately to compare seed sizes and weights. Flowering time could be measured in the primary racemes. The minimum width and maximum thickness of 50 seeds each of Minibean, Maxi and Hamburg were measured with an electronic calliper; the weight of each of these seeds also was determined. Analyses of variance were conducted on the mean values per plant for each pot.

Results

Most seeds of Maxi were larger, and most Minibean seeds were smaller, than 10 mm in diameter. However, environmental conditions during seed filling also markedly affect seed size distributions. Thus, Maxi grown in the ACT in 1995 had 85% by weight of its seed above 10 mm diameter, but only 58% attained that size in 1994, when conditions were much hotter and drier. Similarly, the percentages of seed of Minibean below 10 mm diameter were 97 in 1994 and 90 in 1995. The yields of Maxi and Minibean in different isolated seed-increase areas in the 1994 drought year were 1.20 and 0.71 t/ha, whereas in 1995, the respective yields were 2.87 and 3.49 t/ha.

The yields and seed sizes of these new cultivars at Wagga Wagga and Cowra were compared with those of current cultivars and some candidates for release through the national breeding program (Table 1). Although Maxi seeds had a lower average diameter than those of A75Bo9-2-3, the mean seed weight of Maxi (465 **±**13 mg) was non-significantly larger than that of A75Bo9-2-3 (448 ±13 mg). The alkaloid concentrations in four seed samples each of Minibean and Maxi from the core trials were in the range 0.002-0.015%. These values are within the limit of 0.02% for low alkaloid cultivars.

Table 2 shows that Minibean and Maxi had intermediate flowering times, with Kiev Mutant and Ultra earlier, and Hamburg, Esta, and Multolupa later flowering.? Maxi was slightly later than Minibean. Leaf numbers on the main stem were correlated with flowering date ($r=0.77$, $P<0.05$), whereas the length of the primary stem was not correlated with leaf number ($r=0.24$, $P<0.1$) and only loosely associated with flowering date (r=0.61, P=0.10). Minibean and Hamburg had a shorter primary stem than the early-flowering cultivars, Kiev Mutant and Ultra.

Minibean had the lightest seeds, especially in the lowest fertile order of flowers, the secondaries (Table 2). The mean seed weight of Maxi was not significantly different from that of the other lines except Minibean, but it had a higher percentage of its total yield in the lowermost order of fertile flowers. Minibean was intermediate in this respect. The mean minimum diameters of the seeds of Minibean, Maxi and Hamburg were 9.56±0.09, 10.48±0.09 and 10.95±0.09 mm, respectively, and the corresponding mean maximum thicknesses were 5.73**±**0.05, 6.23±0.05 and 6.00±0.06 mm. The weights per seed of Minibean, Maxi and Hamburg were 426**±**9.5 mg, 569±8.0 mg and 571±11.9 mg, respectively.

Discussion

Minibean yielded well in 1995 and 1996 at Cowra and Wagga Wagga (Table 1), but its mean over four sites in the Riverina in 1995 was a few per cent below that of the highest-yielding line, Multolupa (8). In 1996, Minibean averaged 2% less over 12 sites than the top-yielding lines, Kiev Mutant and 75Bo9-2-3. During the last year of selection at Canberra, Minibean yielded 4.4 t/ha, apparently because of the unusually dense stand resulting from its small seed size. Maxi was lower yielding than the other lines at most sites in each year, but was the highest yielding line at Galong, a cooler, more elevated site with the highest mean yield. This result is not surprising, because the cultivar was bred in even milder ripening conditions at Canberra. Both cultivars may find a place in the crop rotations of such environments.

Extensive genotype x environment interaction in yield was also found by Green (4), who grew 20 accessions, cultivars and breeding lines at Canberra and Wagga Wagga in 1975, 1976 and 1977. The genotype x environment component of variance in yield was considerably greater than that for genotype, with the genotype x year component much larger than the genotype x location component. The principal causes of the differences in genotype ranking in the six different environments were variations in the length of the growing season, in the prevalence of weeds, and in the severity of the brown leaf spot disease and of manganese toxicity. It has proven possible to minimise the effects of these limiting factors under experimental conditions for over a decade in Canberra by following a rotation of lupins, barley, Indian mustard/canola and one-year phalaris, and by the addition of 2.5 t/ha of lime about every tenth year to eliminate manganese toxicity. The brassica crop appears to reduce the incidence of fungal root rots in the lupins. Weed control is assisted by the alternation of broad-leafed and gramineous crops.

The seed size of Minibean was consistently smaller than that of the other lines at Cowra, Wagga Wagga (Table 1) and at Canberra, and Maxi had a higher proportion of large seeds than all other lines except A75Bo9-2-3. However, the latter two lines had similar mean seed weights, and Maxi seeds were significantly thicker than those of A75Bo9-2-3. The plumpness of the seeds of Maxi may well reflect the higher proportion of seeds which it bears in the lowermost order of pods.? Even in the extremely favourable cool, moist spring of 1995 in the A.C.T., Maxi produced most of its seeds in the first and second orders of pods. The concentration of weight in the second order pods caused many primary stems to split at the point of insertion of the branches during a rain storm. Nevertheless, the seeds on the branches of these plants appeared to mature normally.? The proportion of large seeds in the Esta samples also was high, and not significantly lower than the proportion in Maxi. Green (4) found that the genetic component of variance in seed weight was about nine times greater than the genotype x environment component, thus showing that selection for seed size would be much more effective than selection for yield, and that the differences between lines with contrasting seed sizes would be relatively stable across environments. Indeed, very large-seeded, high alkaloid cultivars of the 'Lupini Bean' type have been grown in Italy for many centuries, and a small-seeded cultivar, Kleink?rnige, was registered in West Germany in 1950 (1).

The composition of white lupin seed is? favourable for human and animal nutrition. Whole seeds contain 33-42% crude protein in which there are adequate amounts of the amino acids essential for non-ruminant animals except for those containing sulfur, methionine and cysteine, which total only 2.2% (5). This deficiency is unimportant in mixtures with cereals, which contain an excess of these amino acids. Also, the protein contains enough lysine and threonine to counteract the deficiencies of these amino acids in cereals (5). The oil content of 8.3-11.3% contributes significantly to the metabolisable energy content of mixed diets, but the principal storage carbohydrate,?<F"Symbol"b-(1<F"Symbol"?4)-galactan (7), is digested slowly by pigs, thus reducing their daily food intake and growth rate. The proportions of oleic acid (44-54%), linoleic acid (17-27%) and linolenic acid (6.7-15.2%) in the oil are favourable for arterial health in humans, but the level of the harmful saturated fatty acid, palmitic, is 5.4-9.4% (5), which is twice that of canola oil.

Several improved *L. albus* cultivars may be released soon in eastern Australia. Certified seed of Minibean and Maxi has been produced by Mr David Jacobs, Wallendbeen and Mr Bruce Klimpsch, Mangoplah, respectively, in 1996 and 1997. Esta is also being seed increased for possible release because of its improved tolerance to *Pleiochaeta* root rot. Furthermore, A75Bo9-2-3 may be released as a large-seeded cultivar (D. J. Luckett, *pers. comm*.). The second author and Dr Luckett have selected low alkaloid segregates with seeds approaching 15 mm in diameter from crosses between the high alkaloid 'Lupini bean' type and Australian genotypes. These should provide the next generation of large-seeded cultivars.

One unfavourable feature of all these nascent cultivars is their susceptibility to race 2 of the anthracnose fungus, *Colletotrichum gloeosporioides* Penz. & Sacc., which first damaged white lupin crops in W.A. in 1996.? Resistant, late-maturing, Ethiopian lines have been discovered (9), but their agronomic potential in eastern Australia has not yet been evaluated. This race is common on Russell lupin seedlings because they are grown by nurserymen from imported infected seed in all states.? The disease probably will not persist in home gardens if nurserymen use healthy seed. Also the periods between highly susceptible lupin crops in eastern Australian rotations are much longer those in W.A., so that outbreaks in the east are likely to affect only scattered paddocks, with little persistence from year to year (G. Murray, *pers. comm*.).

Conclusions

The availability of long-season cultivars of lupins with contrasting seed sizes will meet a need for high-yielding leguminous crops in the cooler, higher rainfall Tablelands and Slopes districts where high-yielding crops of winter wheat, triticale and canola are increasingly being grown to complement the traditional wool and meat production industries. The ruminant stockfeed market should accommodate a much larger output of lupin seed than the present small levels. The higher value, export, whole-seed food market currently is limited to about 20,000 t/annum, but sales of split seeds and flour, as produced at Cootamundra by Crokers, should have more potential in Australia and overseas as both food and feed.

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