IMPROVED HERBICIDE TOLERANCE IN NARROW-LEAFED LUPIN (*LUPINUS ANGUSTIFOLIUS* L.) THROUGH UTILIZING NATURAL VARIATION AND INDUCED MUTANTS

Ping Si[1], John Quealy[1], Mark Sweetingham[2] and Bevan Buirchell[2]

[1]Centre for Legumes in Mediterranean Agriculture, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia
[2]Department of Agriculture and Food, Western Australia, Locked Bag 4, Bentley Delivery Centre, WA 6983, Australia

Corresponding authors email: pingsi@cyllene.uwa.edu.au

ABSTRACT

The minimum tillage farming systems in Western Australia require cultivars with herbicide tolerance for the effective weed control in crops. Thus, improving herbicide tolerance is one of the breeding objectives of the narrow-leafed lupin (*Lupinus angustifolius* L.) breeding program. We have successfully improved narrow-leafed lupin with tolerance to post-emergent metribuzin. Germplasm screening revealed that there were tolerant and susceptible commercial cultivars, advanced breeding lines and accessions in the germplasm collections. The differences between the tolerant and the very susceptible varieties to metribuzin at seedling stage were large. Tolerant plants showed no foliar damage whilst the very susceptible plants died. Selection for tolerance in an advanced breeding line (WALAN 2173) of a mixture of tolerant and very susceptible plants in 2003 led to the release of cv. Coromup in 2006 with improved metribuzin tolerance. Mutation breeding also proves effective in improving the tolerance. Two mutants (Tanjil-AZ-33 and Tanjil-AZ-55) induced from a metribuzin susceptible, but Anthracnose resistant cultivar Tanjil showed 6 fold increases in tolerance than the original cv. Tanjil. They also had higher tolerance to metribuzin than the tolerant cultivar Mandelup, which is widely grown. These two mutants maintained the high resistance to Anthracnose found in cv. Tanjil. Tanjil-AZ-33 had twice the seed yield of cv. Tanjil in presence of metribuzin. Mutant Tanjil-AZ-33 has become a new source of metribuzin tolerance and Anthracnose resistance.

KEYWORDS

Lupinus angustifolius, metribuzin tolerance, herbicide tolerance, induced mutants, germplasm

INTRODUCTION

Narrow-leafed lupin (*Lupinus angustifolius* L.) is a major grain legume crop in Western Australia (WA). Under the current lupin production systems in Australia, the top agronomic issues are weed and disease management control. Cultivars with increased tolerance to herbicides are needed to expand weed management options in the minimum tillage farming systems. The Anthracnose resistant cultivar Tanjil has been widely used as a parent in the lupin breeding program in Australia as a source of Anthracnose resistance. Unfortunately, it was found also to be susceptible to metribuzin herbicide (Si *et al.* 2006a). Therefore, identification of genotypes tolerant to metribuzin and incorporation of the tolerance into Anthracnose resistant cultivars becomes critical for lupin production.

Two approaches of screening of germplasm across cultivars, breeding lines and core collection and inducing mutation in the Anthracnose resistant, metribuzin susceptible cv. Tanjil have been used in parallel to identify tolerant genotypes. Tolerance exists in the germplasm (Si *et al.* 2006a) and greater tolerance was found in induced mutants (Si *et al.* 2006b). This paper reports the utilisation of natural variation for improvement of metribuzin tolerance and the main features of two highly tolerant mutants in terms of metribuzin LD_{50} and seed yield.

MATERIALS AND METHODS

ASSESSING VARIATION IN GERMPLASM

Plants of cultivars, advanced breeding lines and core collection of narrow-leafed lupin germplasm were examined for their tolerance to post-emergent metribuzin in pot experiments under controlled temperature in a phytotron at 20/12°C in 2002 and 2003, unless otherwise specified. Lupin plants at the 4 leaf stage were sprayed with metribuzin at 200 g/ha in a spray chamber. Details of experimental designs, growing conditions, herbicide application and measurements were the same as described in Si *et al.* 2006a.

CHARACTERISATION OF TOLERANT MUTANTS

Two metribuzin tolerant mutants, Tanjil-AZ-33 and Tanjil-AZ-55 (Si *et al.* 2006b), along with metribuzin-sensitive cv. Tanjil plants (the original parent of the

IN J.A. Palta and J.B. Berger (eds). 2008. 'Lupins for Health and Wealth' Proceedings of the 12th International Lupin Conference, 14-18 Sept. 2008, Fremantle, Western Australia. International Lupin Association, Canterbury, New Zealand. ISBN 0-86476-153-8.

mutants) and metribuzin tolerant cv. Mandelup plants were examined in a dose response study involved 8 doses ranged from 0 to 6400 g/ha, 4 replicates and 20 plants per replicate. Experimental procedures were the same as reported in Si *et al.* 2006a.

The two mutants were compared with cultivars of Tanjil and Mandelup for seed yield when 300 g/ha metribuzin was applied to seedlings at 6 leaf stage with boom spray fitted on a motorbike with output of 72 L/ha. Plants were grown in 3.6 m2 (1.5 x 2.4) plots with a seeding rate of 50 seeds/m^2. Plants from each plot were harvested by hand at maturity and seed yield obtained. Anthracnose resistance of the mutants was assessed in the disease nursery along with other breeding lines and control cultivars in 2007 (Si *et al.* 2008).

RESULTS AND DISCUSSION

UTILIZING NATURAL VARIATION

Lupin cultivars were grouped into three categories of tolerant, susceptible and very susceptible, according to their plant damage scores at the rate of 200 g/ha metribuzin (Table 1). Tolerant cultivars of Mandelup, Belara and Kalya had no or little symptoms of leaf damage and these cultivars are safe with metribuzin applied at the recommended rate in the field for the control of broadleaf weeds. The very susceptible cultivars Chittick and Danja showed severe damage and most plants died. The susceptible cultivars Tanjil, Quilinock, and Wonga suffered moderate leaf scorches in most plants and they would suffer yield loss as a result of the herbicide application.

Table 1. Cultivars of narrow-leafed lupin in response to metribuzin at 200 g/ha at 20/12^{o}C temperatures.

Category	Range of plant score[A]	Cultivar
Tolerant	0-0.9	Belara, Gungurru, Jindalee, Kalya, Mandelup, Marri, Merrit, Myallie, Tallerack, Warrah, Yorrel
Susceptible	1.0-3.0	Illyarrie, Moonah, Quilinock, Tanjil, Wonga, Yandee
Very susceptible	3.1-5.0	Chittick, Danja, Fest, Geebung, Unicrop, Uniharvest, Uniwhite

[A]Scale of leaf score is between 0 and 5, where 0 indicates no symptoms and 5 indicates defoliated or dead plants.

Table 2. Genotypic variation in response to metribuzin at 200 g/ha in lupin seedlings grown outdoor during June-July 2003.

Genotype	Plant score [A]		Survival (%)	Shoot DW (% control)	Height (% control)	Leaf area (% control)	Leaf number (% control)
	Mean	Range					
Cultivar							
Belara	0.9	0-2.1	100	68	86	78	81
Kalya	0.6	0-1.7	100	74	91	82	84
Mandelup	0.8	0-1.7	100	63	68	74	68
Tanjil	3.8	3-5	90	38	57	12	31
Breeding line							
95L206-12-14	4.7	3-5	38	36	66	4	23
95L208-13-13	0.2	0-0.7	100	84	88	88	89
WALAN 2127	4.2	3.7-5	77	29	54	6	26
WALAN 2139	0.8	0-1.7	100	69	75	76	78
WALAN 2147	4.4	3.7-5	44	31	57	5	32
WALAN 2173	1.9	0-5	81	55	65	52	74
WALAN 2189	0.7	0.3-1.7	100	69	73	69	71
LSD (P = 0.05)	0.8		16	13	13	18	14

[A]Scale of leaf score is between 0 and 5, where 0 is of no symptom and 5 is leaves defoliated or a plant dead. Presented in the table is mean of first 4 leaves.

Table 3. Seed yield of mutants and cultivars subject to 300 g/ha metribuzin in the field at Shenton Park in 2006 and Anthracnose resistance score in disease nursery in absence of metribuzin in 2007.

Genotype	Seed yield (t/ha)	Visual damage score against metribuzin[A]	Anthracnose resistance rating[B]
Tanjil-AZ33	4.23	0	6.8
Tanjil-AZ55	2.62	0	6.8
Tanjil	1.86	3	7.2
Mandelup	4.63	0	6.1
LSD (P = 0.05)	*1.19*		*1.2*

[A]0 = no symptom; 3 = most plants had scorch on first 6 leaves; 5 = plant dead.
[B]1 = plants severely damaged; 9 = plant immune

The core collection of germplasm exhibited similar range of variation in tolerance. Most advanced breeding lines were either very susceptible or tolerant. Only the tolerant lines were selected and progressed further in the breeding program. Susceptibility to metribuzin in advanced breeding lines reiterates the importance of selection for metribuzin tolerance concurrently with disease resistance in the lupin breeding program. This is particularly important as Tanjil has been used widely as a source of resistance to Anthracnose disease.

SELECTED GENOTYPES UNDER 2 ENVIRONMENTS

A number of cultivars and advanced breeding lines were further examined in both the absence and the presence of metribuzin in a 20/12°C phytotron and under winter outdoor environments. In comparison with plants grown in absence of metribuzin, tolerant plants showing few symptoms of leaf damage still had 15 to 20% shoot dry weight reduction over the course of two weeks after herbicide application (Table 2). Cultivars and breeding lines across these two environments were consistently tolerant or susceptible. One advanced breeding line WALAN 2173 had leaf score showing susceptibility (Table 2). Close examination of the individual plants revealed about 70% of plants had no leaf damage while about 19% plants were killed (i.e. 100 – 81% survival). The large differences between plants within this breeding line were consistent across two environments. Individual plants of the other genotypes were uniform. Selection of tolerance within WALAN 2173 would make this line become uniformly tolerant.

SELECTION FOR TOLERANT PLANTS IN THE ADVANCED BREEDING LINE (WALAN 2173)

WALAN 2173 has higher protein content than other lines and was released in 2006 as cultivar Coromup with improved tolerance to metribuzin. The improved tolerance resulted from selection for tolerant plants within WALAN 2173 in June 2003. Approximately 2000 plants at the 4 leaf stage were sprayed with metribuzin at 200 g/ha and 500 plants with no symptom of damage were selected and transplanted into the field to grow to maturity. The selected plants were bulk-harvested and multiplied again over summer at Manjimup. This seed stock became the source for seed multiplication of cv. Coromup. The level of metribuzin tolerance in cv. Coromup is the same as cv. Mandelup.

INDUCED MUTANTS FROM THE METRIBUZIN SUSCEPTIBLE CV TANJIL

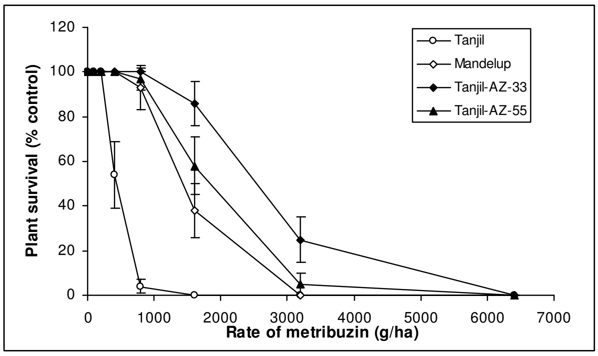

Fig. 1. Percent plant survival of induced mutants Tanjil-AZ-33 (♦), Tanjil-AZ-55 (▲) compared with original parent cv. Tanjil (○) and a tolerant cv. Mandelup (◊) in metribuzin dose responses with plants grown in a 20/12°C (day/night) phytotron.

The tolerance level of mutant Tanjil-AZ-33 was 6 times greater than cv. Tanjil by measure of LD_{50} (Fig. 1). At the metribuzin rate of 800 g/ha, all Tanjil plants died whilst the two mutants of Tanjil-AZ-33 and Tanjil-Az-55 had 100% survival. The mutant of Tanjil-AZ-33 showed greater tolerance (higher survival rate) than Mandelup at rates greater than 1600 g/ha. Tanjil-AZ-33 is the most tolerant genotype known in lupin germplasm. This fact reconfirms that induced mutation creates new tolerance. Mutation breeding has been successfully used in soybean for increased tolerance to sulfonylurea herbicide (Sebastian and Chaleff, 1987). Cultivars with greater tolerance than Mandelup are preferred for the WA farming systems as they could potentially lead to higher application rates to allow more effective control of weeds. These mutants are valuable sources for the development of lupin cultivars with greater tolerance.

Seed yield of mutant Tanjil-AZ-33 was 4.23 t/ha under irrigation, more than twice that of Tanjil when they were subject to metribuzin at 300 g/ha during the 6-8 leaf stage, but very close to that of the tolerant and high yielding cultivar Mandelup (Table 3). Both mutants showed no symptoms of leaf damage from metribuzin at 3 weeks after application, whilst Tanjil plants were severely damaged. It is expected that new cultivars combined with the high yielding background of Mandelup and high metribuzin tolerance of Tanjil-AZ-33 would be able to tolerate higher rates of herbicide and hence have higher yield.

Tanjil-AZ-33 and Tanjil-AZ-55 had similar Anthracnose resistance scores as Tanjil in a disease nursery in 2007 (Table 2), indicating that they were as highly resistance to Anthracnose as Tanjil. The two mutants were selected among several other tolerant mutants on the basis of their carrying the molecular marker of Anthracnose resistant gene (Si *et al.* 2006b). Presence of the specific molecular marker for Tanjil's resistance to Anthracnose in all seedlings of the two mutants suggests that these mutants retained the Anthracnose resistant gene (You *et al.* 2005). We hoped that some mutants would retain the Anthracnose resistance of Tanjil but improvement in tolerance to metribuzin. Anthracnose assessment in the disease nursery confirmed the molecular marker assessment.

Induced mutation in cv. Tanjil has created new mutants with increased tolerance to metribuzin, but maintained high resistance to Anthracnose, the highly desirable trait of Tanjil. These mutants prove to be a parental source of high tolerance/resistance to metribuzin and Anthracnose. Tanjil-AZ-33 has been used as a parent by lupin breeders in a cross involving Mandelup and Coromup, in an effort to combine the features of metribuzin tolerance, Anthracnose resistance, high yield and high protein content into one cultivar.

ACKNOWLEDGEMENTS

This research is funded by Grains Research and Development Corporation. We are grateful to the lupin breeding staff at DAFWA for their technical support.

REFERENCES

Sebastian, SA. and R.S. Chaleff. 1987. Soybean mutants with increased tolerance for sulfonylurea herbicides. Crop Science 27: 948-952.

Si, P., M.W. Sweetingham, B. Buirchell, D. Bowran and T. Piper. 2006a. Genotypic variation on metribuzin tolerance in narrow-leafed lupin (*Lupinus angustifolius* L.). Australian Journal of Experimental Agriculture 46: 85-91.

Si, P., M. Sweetingham, B. Buirchell and H. Yang. 2006b. Lupin mutants highly tolerant to metribuzin. Proceedings of 13th Australian Plant Breeding Conference, 18-21 April 2006, Christchurch, New Zealand. C.F. Mercer (ed), pp. 326-329.

Si, P., B. Buirchell and M. Sweetingham. 2008. Induced mutation in narrow-leafed lupin improvement: an example of herbicide tolerance. In proceedings of FAO/IAEA International Symposium on Induced Mutation in Plants, 12-15 August 2008, Vienna, Austria.

You, M., J.G. Boersma, B.J. Buirchell, M.W. Sweetingham, K.H.M. Siddique and H. Yang. 2005. A PCR-based molecular marker applicable for marker-assisted selection for Anthracnose disease resistance in lupin breeding. Cellular and Molecular Biology Letters 10: 123-134.