Genotypic differences for drought resistance in *Lablab purpureus* L.

Cristine Hall[1] and B.P. Naidu[1,2]

[1] CSIRO Sustainable Ecosystems, Toowoomba, Qld 4350. cristine.hall@csiro.au
[2] Present address: Departments of Natural Resources, Mines & Energy, and The University of Queensland (SLFS),
80 Meiers Rd, Indooroopilly, 4068.

Abstract

Fifteen genotypes of lablab (*Lablab purpureus* L.*)*, including commercial cultivars, cultivated and wild type accessions, and bred lines were evaluated for drought resistance in a controlled environment pot experiment. Drought resistance was not related to whether a genotype is annual or perennial. One of 2 commercial cultivars, 4 of 8 cultivated accessions, 1 of 3 wild accessions and 2 of 2 bred lines were measured as drought resistant after a prolonged period of withholding water. This study demonstrated that there is a high level of variation for drought resistance within the lablab genotypes and that with the extensive germplasm available, genotypes with the desirable characteristic of drought resistance could be selected to enhance forage lablab breeding programs that already focus on high yield and perenniality.

Media summary

A drought resistant, perennial and high yielding forage lablab cultivar is the potential outcome of a continued breeding program combining traditional and molecular plant breeding science.

Key words

Lablab purpureus, drought resistance, water use, relative water content.

Introduction

Water deficiency is a major factor that reduces the potential productivity of forage plants in the tropics. Different mechanisms contribute to drought resistance in plants. These include the avoidance of plant water deficits by drought escape (short duration) , water conservation, and more efficient water uptake (Jones, 1983). More than one of these mechanisms can be used as a defence strategy by plants.

Most annual plants adapt their growing cycle to finish their reproductive phase before the period of drought (Monneveux and Belhassen, 1996), while perennial species tend to develop efficient organs (deep tap roots, water reservoirs) or physiological mechanisms. Plant breeding programs have researched the mechanisms of drought resistance in many species, but drought continues to affect crop production and challenge plant breeders (Ceccarelli and Grando, 1996).

Lablab (*Lablab purpureus* L.) is a fast growing tropical summer multi-purpose legume suitable for ley farming and forage systems in Australia. Lablab originated in Africa and is now cultivated in tropical countries around the world. In India and Africa it is grown as a grain legume crop. Most of the commercial cultivars and the cultivated accessions are annuals, while a majority of wild types are perennials. There are some bred lines, which are weak perennials.

The objectives of this study were a) to identify genotypic differences for drought resistance in a range of lablab genotypes and to verify if drought resistance is confined to any particular habit (annual or perennial) of lablab, and b) to examine if drought resistant genotypes could be selected under laboratory conditions based on some simple physiological attributes.

Materials and Methods

Sixty pots (20 cm diameter by 80 cm high cylinders) lined with plastic were each filled with 13 kg of fertile loam soil and watered with 2 litres of water. Soil surface was covered with plastic beads to prevent evaporative loss of water. Four replications of 15 lablab genotypes (Table 1) were planted from germinated seed. Eight seedlings per pot were established and subsequently reduced to four plants. The experiment was conducted in a controlled environment room, with 30/25 ?C day/night temperature and 14 h photoperiod. The relative humidity was 60 and 90%, during day and night, respectively.

Pots were watered to weight (total 15 kg) for four weeks by which time, the plants had grown to at least 20 cm in height. The first fully expanded leaf was sampled from one plant of each pot to measure % relative water content-initial (RWC-initial): [(fresh wt-dry wt)/ (turgid wt-dry wt)] 100.

At 4 weeks after sowing, pots were watered for the final time to 15 kg and drought stress was imposed by withholding water. Water use was monitored by weighing the pots and ranked (from highest to lowest water use) at 5, 9 and 12 days after imposing water stress. No change in water use was noted at the end of 12 days and it was considered that plants used up most of the available water during this period. At day 12 into the drought stress regime, another leaf sampling was done to calculate and rank RWC during drought stress (RWC-stress). The decline in RWC (RWC-decline) was calculated as the difference between RWC-initial and RWC-stress and rank (from highest to lowest) in each genotype at the end of the 12-day stress period.

At 9 weeks after sowing (5 weeks after withholding water or 3 weeks after most of the applied water was used by the plants), a visual assessment of foliage yield was recorded (Growth Rating). A rating of 4 indicated a high percentage of green leaf, minimal leaf loss, good vigour and minimal turgor loss (visible wilting) in the leaves and a rating of 1 indicated the lowest value for these parameters.

Leaves that died from each pot throughout the imposed drought regime were collected and weighed. At 12 weeks after sowing (8 weeks after the start of drought stress or 6 weeks after most of the water has been used), 50% of the lines appeared dead. At this time, all pots were re-watered and within 2 days after re-watering, surviving green leaves were harvested and dry wt recorded. Leaf survival % was calculated: [Green leaf wt / (green +dead leaf wt)] 100. The level of recovery and re-growth was monitored 2 weeks after re-watering and was used as an indicator of drought resistance and ranked from the highest to the lowest.

Results

There were significant differences ($P < 0.05$) in the total water use among the 15 lablab lines at days 5, 9 and 12 after the beginning of drought stress. Highworth used water at the fastest rate to day 5 after withholding water (Table 1). When there was less water available at day 9, the water usage rate by all genotypes declined, however, CPI 29803 used water at the fastest rate, followed by cv. Highworth. At day 12, CPI 29803 had used the most water, followed by cv. Highworth, CPI 24973 and CPI 34782. When water was mostly unlimited, over 5 days after withholding water, there was a positive but non-significant correlation (r=0.3) between water use and growth ratings. However, no such relationship was apparent at days 9 and 12, when water availability would have been declining.

Table 1: Total water use over 12 days after imposing drought stress by withholding water and growth ratings at 9 weeks in lablab genotypes.

Genotype?	Type?	Habit	Water use (WU, Litres) days after withholding water			Growth at 9 weeks after sowing
			5 Days	9 Days	12 Days	

			WU	Rank	WU	Rank	WU	Rank	Rating	Rank
		Annual	1.06	1	1.62	2	1.73	2	3.75	2
Rongai	Cultivar	Annual	0.74	11	1.37	12	1.68	11	3.50	4
CPI 29803	Cultivated accession	Annual	0.97	2	1.64	1	1.77	1	2.25	11
CPI 34777	Cultivated accession	Annual	0.64	15	1.31	15	1.66	12	2.00	13
CPI 34781	Cultivated accession	Annual	0.82	7	1.52	5	1.70	6	2.50	10
CPI 34782	Cultivated accession	Annual	0.82	6	1.46	9	1.72	3	3.00	7
CPI 106504	Cultivated accession	Annual	0.70	13	1.32	14	1.65	14	3.75	3
CPI 106548	Cultivated accession	Annual	0.73	12	1.34	13	1.63	15	3.50	5
CQ 3319	Cultivated accession	Annual	0.78	9	1.55	4	1.71	5	2.00	12
P 5309	Cultivated accession	Annual	0.91	4	1.50	7	1.69	9	3.50	6
CPI 24973	Wild collection	Perennial	0.85	5	1.51	6	1.72	4	1.00	15
CPI 60216	Wild collection	Perennial	0.68	14	1.43	10	1.70	7	1.50	14
CPI 51564	Wild collection	Perennial	0.94	3	1.56	3	1.70	8	4.00	1
CSIRO 44	Bred line	Perennial	0.79	8	1.47	8	1.68	10	2.75	9
CSIRO 55	Bred line	Perennial	0.76	10	1.41	11	1.65	13	2.75	8
LSD (P<0.05)			0.13	N/A	0.13	N/A	0.07	N/A	0.62	N/A

At the beginning of the drought treatment, RWC was high in most of the genotypes except in CPI 60216 and P 5309 (Table 2). These genotypes seem to have less control over transpiration, even when water availability was high. RWC declined during 12 days after withholding water in all genotypes. There was no significant correlation between RWC-initial and RWC-stress, and also there was no consistency in genotype ranking between the two times of its measurement (Table 2). Only 8 out of 15 genotypes survived the prolonged water deficit, as measured by >0 % leaf survival and were regarded as drought resistant. CPI 106504 retained almost 62% of leaves, followed by Highworth, which showed 51% of leaf survival. Genotypes with higher leaf survival also recovered well and re-grew as shown by the drought

resistance rankings (Table 2). The growth rating at 9 weeks after sowing (Table 1) was also in some cases consistent with drought resistance rankings (Table 2). For example, the 5 genotypes with the highest growth ratings were ranked the top 5 in drought resistance, although in a different order.

Table 2. Relative water content (RWC), leaf survival, and drought resistance ratings in Lablab genotypes.

Genotype	RWC-initial		RWC-stresses		RWC-decline		Leaf survival %	Drought resistance rankings
?	%	Rank	%	Rank	%	Rank		
Highworth	90.2	1	74.6	4	15.6	3	51.3	2
Rongai	84.0	6	72.8	6	11.2	9	0.0	9
CPI 29803	82.9	7	64.1	15	18.8	1	9.0	6
CPI 34777	81.7	12	69.8	12	12.0	7	0.0	9
CPI 34781	88.1	2	75.1	3	13.0	4	0.0	9
CPI 34782	84.4	3	71.8	9	12.5	5	0.0	9
CPI 106504	82.5	10	72.1	7	10.4	11	61.7	1
CPI 106548	82.7	9	77.3	1	5.4	14	50.1	3
CQ 3319	82.8	8	70.8	10	12.0	6	0.0	9
P 5309	76.3	14	67.0	14	9.4	12	41.2	4
CPI 24973	84.0	4	67.8	13	16.2	2	0.0	9
CPI 60216	76.1	15	77.2	2	-1.1	15	0.0	9
CPI 51564	80.6	13	72.1	8	8.5	13	27.3	5
CSIRO 44	81.8	11	70.0	11	11.7	8	6.2	7
CSIRO 55	84.0	5	73.6	5	10.4	10	4.2	8

LSD (P<0.05)	**6.1**	**N/A**	**5.1**	**N/A**	**7.9**	**N/A**	**9.0**	**N/A**

Discussion

Of the 2 cultivars, only Highworth was drought resistant (as judged from the leaf survival % of >0) (Table 2) as were 4 of the 8 cultivated accessions and 1 of 3 wild types. Both the bred lines (CSIRO 44 and 55) re-grew on re-watering after the drought. These two lines showed a higher level of drought resistance than their two parental lines, cv. Rongai and CPI 24973. The genotypic variability identified in this study is supported by the considerable diversity identified in a lablab collection by Pengelly and Maass (2001). Three of the lines identified as drought resistant in this study, CPI 106548, cv. Highworth and CPI 51564, were identified in a core lablab selection by these authors and were in separate morphological and agronomic groups.

A higher degree of genetic variability has been identified in lablab wild types than in cultivated accessions by Liu (1996). Liu (1996) noted that the drought resistant wild type in this study (CPI 51564) was genetically similar to a non-drought resistant wild type (CPI 24973). However, the present study demonstrated a contrasting drought resistance between these two genotypes (Table 2). This study also identified a greater difference in drought resistance between the cultivated accessions than is suggested by the relatively similar genetic make-up reported by Liu (1996) for this group. These observations suggest that the RAPD technique used by Liu (1996) failed to completely identify the genetic differences between the lablab genotypes.

Fast growing genotypes such as Highworth used water rapidly, probably because of greater transpiring leaf area (data not shown). Measuring RWC is useful in determining resistance to water loss (Ludlow, 1989), but it was not a good indicator of subsequent lablab survival in this experiment. In this study, RWC was measured immediately prior to the imposition of drought and only once under drought condition. Leaves die when they reach a critical RWC. A time-course measurement of RWC and the determination of the critical RWC would have given an indication of the resistance level of the plant and length of time of continual survival.

Of all the visual and physiological measurements of drought resistance, retention of green leaf area seems to be strikingly similar to yield ratings, and plant recovery after re-watering. It is also clear that green leaf retention is not due to drought avoidance by less water use in the surviving genotypes. For example, water use during the first 5 days (Table 1) was fastest in Highworth and slowest in CPI 3477. However, Highworth maintained more than 50 % of leaf weight and CPI 3477 died. These observations suggest that the genotypes, which survived the prolonged drought, might possess a mechanism for protoplasmic dehydration tolerance. Osmoprotectants (Naidu, 1998) have been shown to be responsible for stress and dehydration tolerance in plants. We have, in fact, found a highly significant correlation (r= 0.91) between leaf survival and the accumulation of, yet to be identified, sugar (data not shown) in these genotypes.

Conclusions

The genotypes CPI 106504, Cv. Highworth, CPI 106548, P5309, CPI 51564 and CPI 29803 could be used as parents to contribute drought resistance in a forage lablab breeding program aimed at producing drought resistant, perennial and high yielding cultivars. Unfortunately, the earlier indicators of drought resistance (RWC, total water use and yield) are only limited indicators of drought resistance. These physiological indicators, including leaf survival are laborious and need extensive experimentation. It is highly advantageous and breeding progress will be rapid, if the (unknown) osmoprotectant in lablab is identified and molecular markers are developed based on already available genetic map for lablab (Konduri *et al.* 2000). The genes responsible for the biosynthesis of the osmoprotectant in lablab could be identified for the introduction into more valuable commercial crops, thereby making them more drought tolerant.

Acknowledgements

We thank GRDC for their financial support of this research.

References

Ceccarelli S and Grando S (1996). Drought as a challenge for the plant breeder. Plant Growth Regulation 20, 149-155.

Jones HG (1983). Plants and microclimate: A quantitative approach to environmental plant physiology. Cambridge University Press.

Konduri V, Godwin ID, Liu CJ (2000). Genetic mapping of the *Lablab purpureus* genome suggests the presence of 'cuckoo' gene(s) in this species. Theoretical and Applied Genetics 100: 866-871.

Liu CJ (1996). Genetic diversity and relationships among *Lablab purpureus* genotypes evaluated using RAPD as markers. Euphytica 90: 115-119.

Ludlow MM (1989). Strategies of response to water stress. In: Structural and functional responses to environmental stresses. 269-281. (Eds: Kreeb KH, Richter H & Hinkley TM).

Monneyeux P and Belhassen E (1996). The diversity of drought adaptation in the wide. Plant Growth Regulation 20, 85-92.

Naidu BP **(**1998). Separation of sugars, polyols, proline analogues, and betaines in stressed plant extracts by high performance liquid chromatography and quantification by ultra violet detection. Australian Journal of Plant Physiology 25:793-800.

Pengelly BC and Maass BL (2001). *Lablab purpureus* (L.) Sweet – diversity, potential use and determination of a core collection of this multi-purpose tropical legume. Genetic Resources and Crop Evolution 48: 261-272.