



Seed quality of **Azuki and Kintoki Beans**

**A report for the Rural Industries Research
and Development Corporation**

by Anthony James Hamilton

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Foreword

This research will help prospective growers evaluate whether they can grow azuki and kintoki beans in their region and, if so, suggest an optimum planting date to maximise seed quality. It will also demonstrate to Japanese processors that research is focussed on supplying optimum quality beans.

Murray Darling Basin Water Reforms are driving more efficient water use and a switch to higher value irrigated crops. Two such crops with potential are azuki (commercially grown in Australia) and kintoki beans (commercial trials in 1999/2000 season).

Japanese bean processors now offer grower contracts for high quality azuki beans. The emphasis is on seed quality and a reliable supply. These contracts specify colour (L^*, a^*, b^*) values. To date, there has been limited research to define the environmental factors that affect seed colour. Current agronomic recommendations, such as for time of planting, aim at maximising yield, but are not related to optimising seed quality.

Azuki and kintoki beans were grown in small plot field experiments over three seasons and on farms located near Forbes, Canowindra and Bathurst in central NSW. Colour measurements will be made on these beans and related to agronomic and environmental factors during the growing season.

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Peter Core

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Executive Summary

This report examines the influence of the thermal environment during the growing season on the seed quality of two dry bean crops, azuki (*Vigna angularis* (Willd.) Ohwi & Ohashi) and taishou kintoki a large, red-seeded kidney bean (*Phaseolus vulgaris* L.). Both varieties are used in East Asian foods, primarily as sweetened products. Kintokis are usually consumed as sweetened whole beans (*amanatto*) and azukis are generally made into a sweetened paste (*ahn*) that is made into various products such as confectionery (*wagashi*). Seed quality is of paramount importance for Australian growers wishing to export high value azuki and kintoki to Japan.

Whilst there has been some research in Australia (eg Desborough and Redden 1996, Redden and Tomkins 1996, Falconer and Desborough 1991) about the agronomy of azuki, there has been minimal research about the environmental and agronomic factors affecting seed quality. Kintoki had not been grown commercially in Australia, but growers and buyers were keen to assess the feasibility of producing high quality beans.

Five commercial azuki crops were monitored during 1999/2000 in an attempt to validate the results of previous small plot experiments conducted as part of a PhD thesis by the author. These crops were grown at sites of similar longitude (33°S) but ranging in altitude from 195m (near Condobolin NSW) to 713m (near Bathurst NSW) above sea level.

Seed colour was measured using a Minolta colorimeter. This machine measures the degree of brightness (L^*), redness (a^*) and yellowness (b^*) using internationally standardised C.I.E. $L^*a^*b^*$ colour-space coordinates. Temperature data loggers were placed in each field. The colour values were then related to daily minimum and maximum temperatures. Results from this experiment confirmed that the thermal environment during pod-fill influences azuki seed size and colour. The cooler the air temperatures during pod-fill the brighter, paler and larger are the azukis and duller, darker and larger are the kintokis. These traits are desired by importers and processors.

Thus delaying planting is an effective strategy to improve seed quality. However, delayed planting also results in decreased seed yield. Thus there is a trade-off between planting early for yield and late for quality. Seed quality decision models may assist growers to optimise their choice.

One Japanese company (Ueno Fine Chemicals Ltd) will set contract prices for azuki based on measurable seed quality parameters including seed size and C.I.E. $L^* a^* b^*$ (International Commission on Illumination 1976) colour values. Growers in central NSW now have experimental data and a preliminary seed quality model to estimate the effect of varying their sowing date on these parameters.

A trial shipment to Japan of about 50 tonnes of kintoki grown in Australia during the summer of 2000/2001 is planned to allow further evaluation of taishou kintoki seed quality by several Japanese importers. Thus, a new crop for Australian growers is in its embryonic stages.

1.Introduction

Azuki growers in Australia primarily export their product to Japan. There is a profitable market for high quality seed. However, growers are less able to compete with lower quality azuki seed, imported from China by Japanese buyers, if their seed does not meet stringent quality standards. Seed size and seed testa colour are the most important determinates of quality (Kato *et al* 2000). Currently, before tendering to purchase azuki, samples are sent to Japan for buyers to make a visual colour appraisal. In future, as a condition of acceptance of the crop by processors, it is proposed to include in contracts with growers' threshold values of colour for beans.

Colour is quantified using the C.I.E. L*a*b* colour space system, which was standardised by the International Commission on Illumination (1976), where measurements of brightness (L*-value), degree of redness (a*-value) and degree of yellowness (b*-value) are recorded. Azuki varieties commercially grown in Australia range in colour from pale red to dark red, with optimum colour being defined as having brightness (L*- values) of 25-30 and yellowness (b*-values) of 8-10 (Desborough and Redden 1996). Other measurements of colour, hue (H*) and chroma (C*) are derived from a* and b* values.

Seed quality has been extensively studied in small plot experiments, by the author, as part of a PhD thesis during the summers of 1996/97 to 1998/99. During the summer of 1999/2000, five commercial azuki crops were monitored during the season and the two most important aspects of seed quality, namely seed size and seed colour, were evaluated.

Previous experiments, conducted by the author, showed there was a relationship between seed colour and thermal environment during pod-fill. There was also a relationship between solar radiation and seed colour. To determine whether solar radiation *per se* was affecting seed colour some pods were covered in foil to exclude radiation.

This report shows that results from small plot experiments are applicable to commercial azuki crops grown over a range of thermal environments in central NSW. It also demonstrates the affect of altitude on azuki seed size.

Despite its importance in determining seed quality in these species, there is little quantitative data, under Australian conditions, of the effects on the development of seed colour of crop management. Recent practice in the Lachlan Valley of NSW was to sow azuki from early- to mid-December to achieve maximum yields. Observations suggest that seed colour may be improved by later sowing, possibly as late as early-January. Colour accumulation has been widely studied in fruit of other species (eg. apples, Arakawa 1991, Faragher, 1983; common beans, Gantet *et al* 1993 and 1993a; and, cranberries, Hawker and Stang, 1985).

This report discusses the effects of varying time of planting, from November to February measured in two years, on seed colour of azuki and kintoki. It also relates colour measurements from commercial crops grown at similar latitude but over a range of altitudes, from Bathurst to Condobolin in central NSW to their thermal environment. It is hypothesised that the colour of the testa of seeds of these species will be associated with conditions, in particular the thermal environment, experienced by plants during pod filling.

2. Materials and Methods

On farm validation of the previous three years' work was undertaken on five properties with similar latitudes (33°S) but varying altitudes ranging from Bathurst in the east (altitude 713m) to midway between Condobolin and Forbes, in the Jemalong Irrigation District (J.I.D.), in the west (altitude 210m)(Table 1).

Table 1 Location of on-farm experiments and weather stations.

Grower	Location	Weather Station	Altitude	Latitude	Longitude
Stewart	Bathurst	63005 Bathurst	713m	33 ⁰ 25'S	149 ⁰ 33'E
Farley	Canowindra	65091 Cowra	300m	33 ⁰ 50'S	148 ⁰ 39'E
Barnes	Eugowra 1	65103 Forbes	230m	33 ⁰ 21'S	147 ⁰ 55'E
Wade	Eugowra 2	65103 Forbes	230m	33 ⁰ 21'S	147 ⁰ 55'E
Hamilton	J.I.D.	50052 Condobolin	195m	33 ⁰ 04'S	147 ⁰ 13'E

The farmers are all experienced azuki growers. Each crop was fully irrigated (border check flood irrigation except the Bathurst site which was spray irrigated) with adequate nutrients applied following commercial practice and the appropriate strain of *Rhizobium* used. Weed control and pest control (mainly *Helicoverpa spp.*) were carried out as necessary, in line with industry practices. Azuki (cv erimo) crops were sown, at a target densities of 40 to 50 plants per square metre, on 13th December 1999 at Bathurst, on 2nd January at Canowindra, on 5th January at Eugowra 1, on 13th January at J.I.D. and on 16th January 2000 at Eugowra 2 site.

Temperature data loggers ("Tinyview", Hastings Data Loggers, Port Macquarie) were placed in screens and located in each paddock. They logged screen temperatures hourly, from which daily maxima and minima were calculated. Harvests were taken on four occasions. Square metre quadrat samples were cut at ground level with secateurs, weighed and subsamples partitioned into leaves, stems, flowers and pods, dried in a forced air dryer and weighed. At the final harvest, mean plant height, the number of branches per plant, number of pods and the number of pods whose lower tips were less than 10cm above ground level, were measured on ten plants per quadrat.

At one site (J.I.D.), a random selection of two groups of Azuki plants was made within the field. The pods of one group were covered in silver foil and the pods of the control left uncovered to see whether solar radiation had any influence on seed colour.

After harvest, azuki seeds were separated from the pods, by hand, weighed and 100-seed weight was calculated and seed samples then subjected to colour analyses.

Daily minimum and maximum screen temperatures (from in-field temperature loggers) were compared to long term daily records (more than 30 years) from the nearest Bureau of Meteorology (B.O.M.) station (Figure 1).

In 1998 and 1999 erimo azuki and taishou kintoki seed was analysed using a Minolta CR-310 tristimulus colorimeter fitted with a 50mm aperture (Minolta, Japan). Prior to recording colour measurements, seeds that had been damaged by insects were removed then small seeds were removed by passing samples over a 4mm-slotted screen. The resulting sample represents commercially graded samples that are tested for colour prior to sale.

The meter was calibrated using a white tile provided by the manufacturer. The beans were poured into a steel canister provided by the manufacturer and the machine head placed within the canister

against the beans. The colour meter automatically takes three readings of reflected light, 3 seconds apart and records an average value. The sample was rotated between readings. Nine readings (ie 3 lots of 3 averaged readings) were made for each sample. There was no attempt to place the white hilum away from the machine head. Some of the samples from later times of sowing were small and reliable measurements of colour could not be made. These were not included in the analysis. Reference samples of commercially grown azuki and kintoki were also measured, but not included in the analysis.

In 2000, erimo azuki seed from five commercial farms was measured using both a Minolta CR-310 tristimulus colorimeter with a 50mm aperture and a Minolta CR-200 tristimulus colorimeter with an 8mm aperture. Temperature data loggers were placed in each field. Colour measurements were related to thermal conditions experienced during pod-fill, using regression analyses.

In an attempt to resolve discrepancies between measurements made in Japan and those recorded in 1999, samples from previous experiments were also retested in 2000 using both colorimeters. The protocol used in 1999 for the Minolta CR-310 (50mm aperture) colorimeter was repeated. An attempt was also made to make colour measurements, with this machine, of the seed testa with their hilums turned down, but this was found to be because as some seeds were turned over others would move to expose the white hilums. However, the Minolta CR-200 with the smaller (8mm) aperture, which only reads 3 to 4 seeds at a time, was used to make two sets of readings. The first reading was made with the hilums randomly distributed and the second set where the hilums were placed down (away) from the light source of the meter.

Two groups of azuki seed samples, one whose pods were covered with silver foil during maturation and colour formation to exclude solar radiation and a second control group left uncovered were also analysed in 2000 for seed colour. These readings were also taken with two machines, a Minolta CR-300 and CR-200.

Data were subjected to ANOVA using GENSTAT 5 (Genstat Committee 1988). Linear or quadratic regression was used to correlate colour values with time of sowing (defined as Julian days), mean minimum temperatures and mean solar radiation during pod fill (defined as first visible pod to physiological maturity).

3.Results

The site at Bathurst was much cooler, with both daily maximum and minimum temperatures about 5°C lower, than the other four sites. All sites experienced some days above 35°C but only the Bathurst site had temperatures below 0°C at the end of the season.

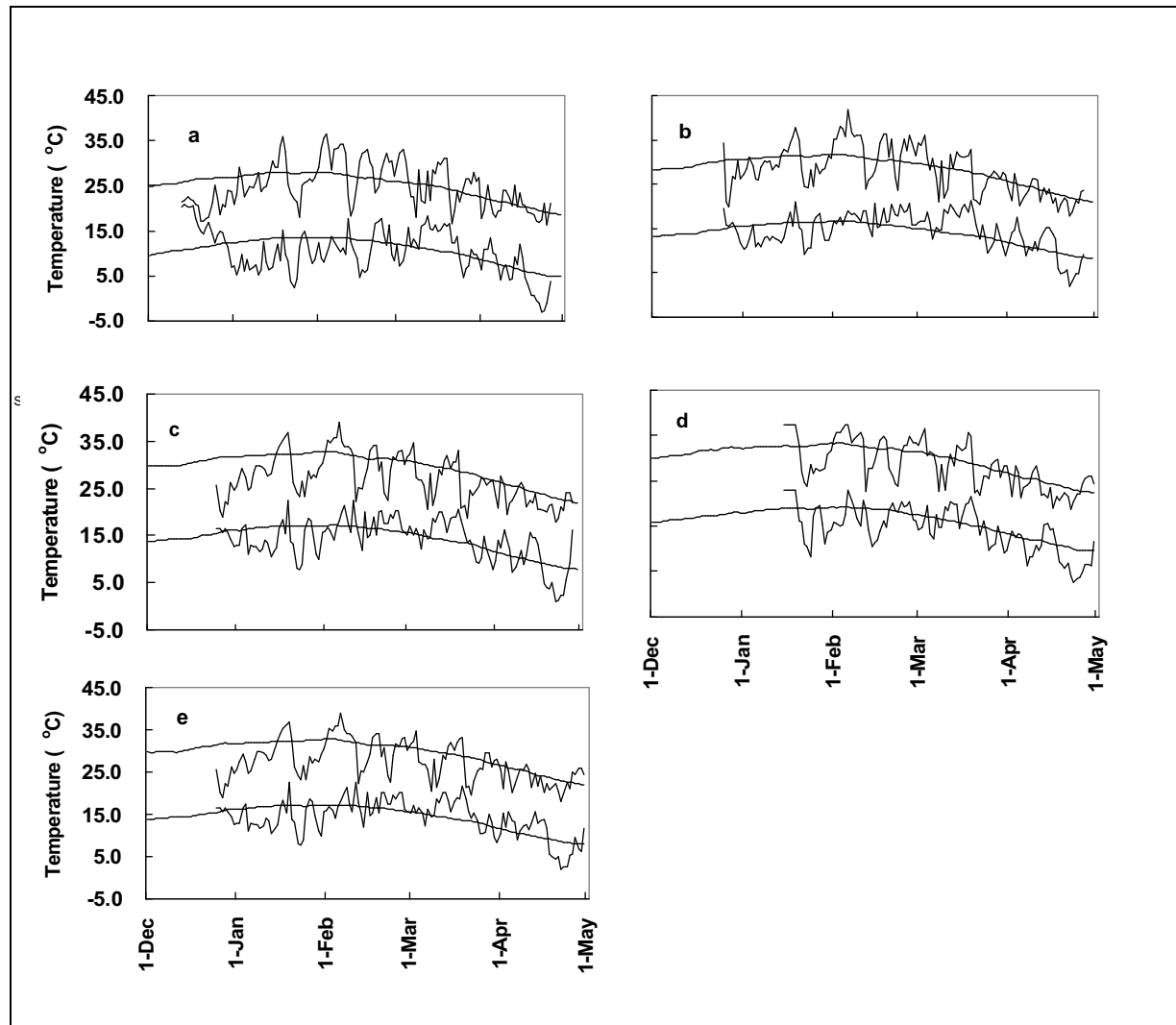


Figure 1 Comparison of daily minimum and maximum temperatures with long-term averages. Long term data (>30yrs) from nearest B.O.M. weather station. 7-day moving averages have been used to smooth long term daily average temperatures. Five sites are Bathurst (a), Canowindra (b), Eugowra 1 (c), Jemalong Irrigation District (d) and Eugowra 2 (e).

Growing degree-days (or heat units) were calculated using a base temperature of 10°C at each location. Thermal time displays an almost linear accumulation (Figure 6.2) over summer until just before maturity when its rate of increase slows considerably in the autumn. Crops planted at the start of January at Canowindra and Eugowra had accumulated more growing degree-days than the Bathurst site by early February and thus flowered before the Bathurst crop, which was sown in mid December. Flowering occurred at about 680 °C.d at all sites.

Even crops planted in mid January matured earlier than the crop sown at Bathurst which was barely reaching maturity when an early heavy frost curtailed growth and accelerated maturity.

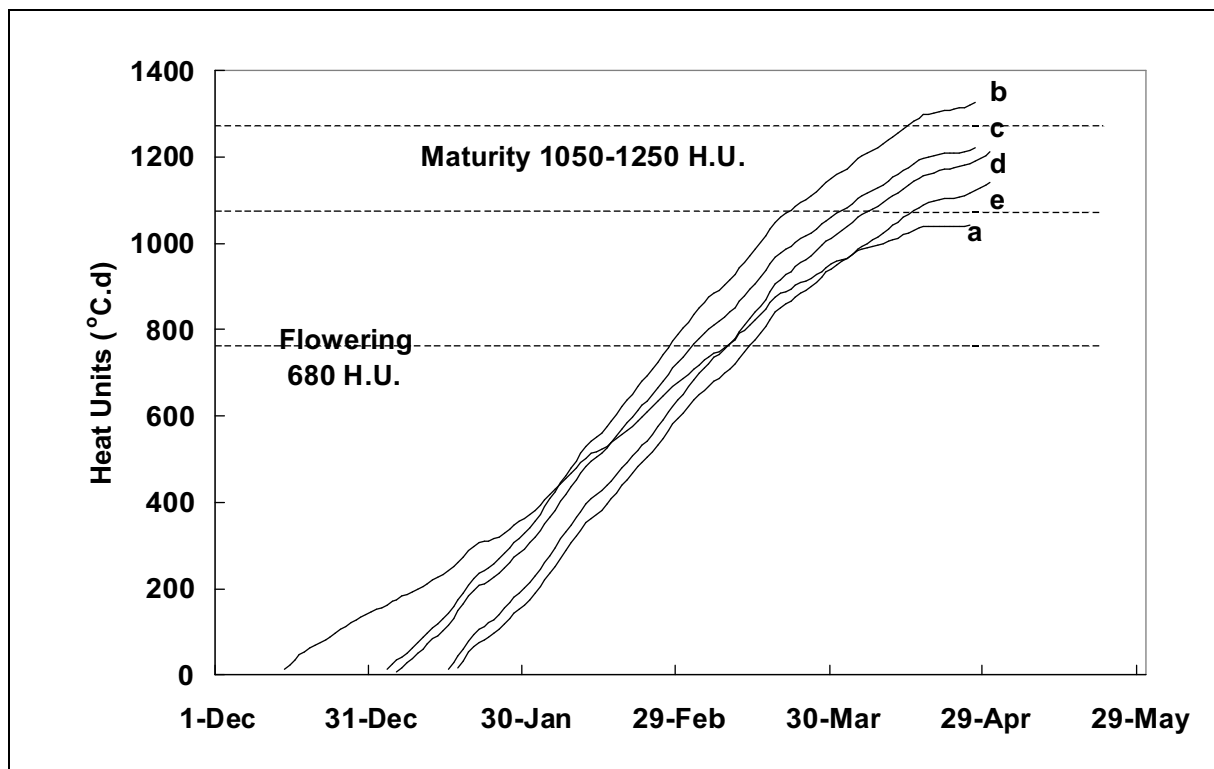


Figure 2 Thermal time (°C.d) graphs for 5 sites (a) Bathurst, (b) Canowindra, (c) Eugowra 1., (d) J.I.D., (e) Eugowra 2., monitored with temperature loggers during the 1999/2000 season. Broken lines show flowering dates (680 °C.d) and maturity dates (ranging from 1050 to 1250 °C.d) for these sites.

Peak above ground biomass ranged from 690 g/m² DM for the latest sown crop (at Eugowra2) to 990 g/m² DM for a crop sown early January (Eugowra1). The earliest sown crop (13th December at Bathurst) had a peak biomass of 790g/m² due to cold weather slowing growth at the end of the season.

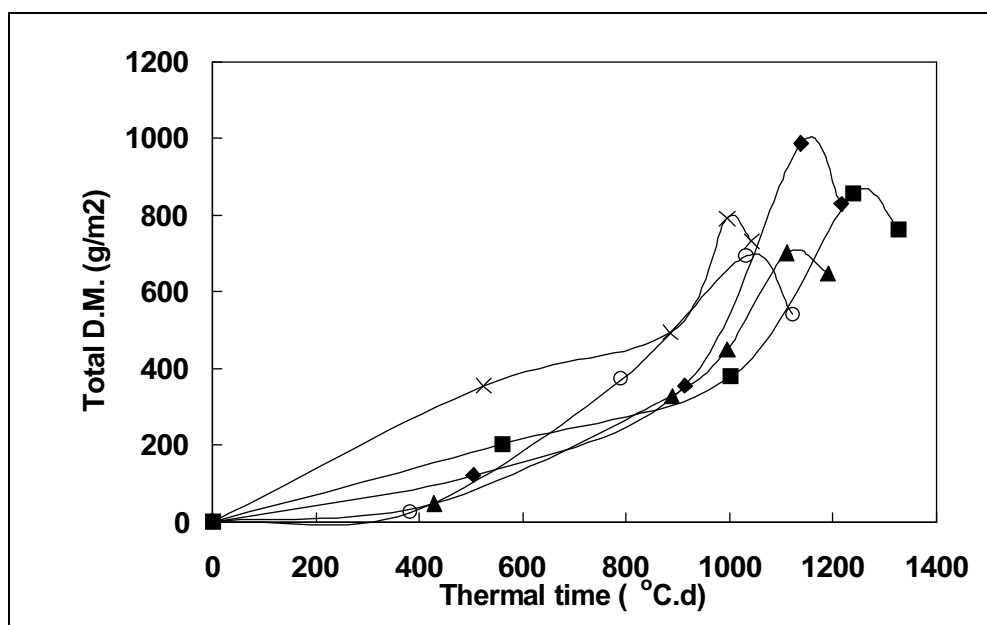


Figure 3 Azuki dry matter production (g/m²) from five sites in central NSW , Bathurst (X), Canowindra (■), Eugowra 1 (◆), Jemalong irrigation District (△) and Eugowra 2 (○), in response to thermal time.

Seed yield was generally a reflection of peak biomass, as the harvest index was relatively constant at about 0.4. Seed yield ranged from 383g/m² to 192g/m² for the latest sown crop (Table 2). Plant height across locations ranged from 45cm to 49cm and the number of branches per plant ranged from 1.8 to 2.7. The number of pods per plant ranged from 12.6 for the earliest sown crop at Bathurst to 18.4 for the latest sown crop at Eugowra2. Despite having the highest number of pods per plant, the crop at the Eugowra2 site had the lowest yield as many pods were small and immature at harvest and it had the lowest plant population density. The number of low pods ranged from 1.3 (10% of pods) to 2.6 (17%) pods per plant.

Table 2 Final harvest and yield components for azukis at five sites

Site	Density Plants/m ²	Total DM (g/m ²)	Seed yield (g/m ²)	Height (cm)	Branches/ plant	Pods/ plant	Lowpods/ plant
Bathurst	41	730	293	48	1.7	12.6	1.6
Canowindra	40	850	383	46	2.3	16.9	2.4
Eugowra1	48	920	368	47	1.8	15.0	2.6
Eugowra2	34	540	192	49	2.7	18.4	2.0
J.I.D.	35	650	252	44	2.0	13.2	1.3
mean	40	740	297	47	2.1	15.2	2.0

Despite all crops being sown at target densities of 40 to 50 plants per square metre only 3 of the 5 crops achieved these densities at harvest time. Final plant numbers ranged from 34 to 48 plants/m² (Figure 4). There was a positive linear relationship between azuki plant population density and above ground biomass ($R^2=0.82$) and seed yield ($R^2=0.63$) at harvest.

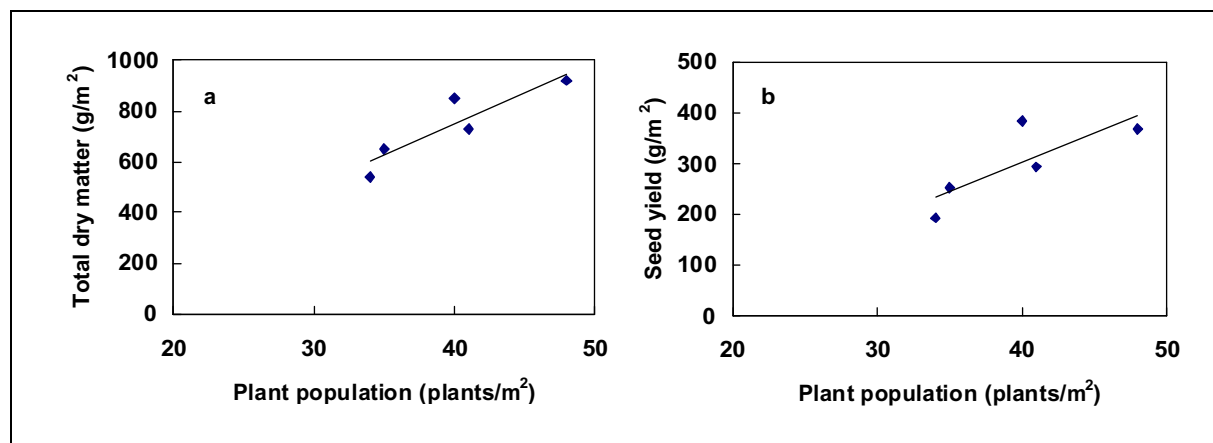


Figure 4 The effect of plant population density on azuki biomass (a) and seed yield (b) from crops grown at five locations in central west NSW.

The cooler the thermal environment during pod-fill, the larger the seed in this experiment. Thus, there was a curvilinear relationship ($R^2=0.92$) between altitude and seed size, ranging from the smallest seed (140mg) grown at the lowest altitude (195m AHD) to the largest seed (180mg) grown at the highest altitude (713m AHD). However, there was not as close agreement ($R^2=0.72$), as there was with altitude, between mean minimum temperature during the last three weeks of pod-fill and seed size. (Figure 5). Seed size was larger in four of five commercial crops than was predicted from previous experimental work.

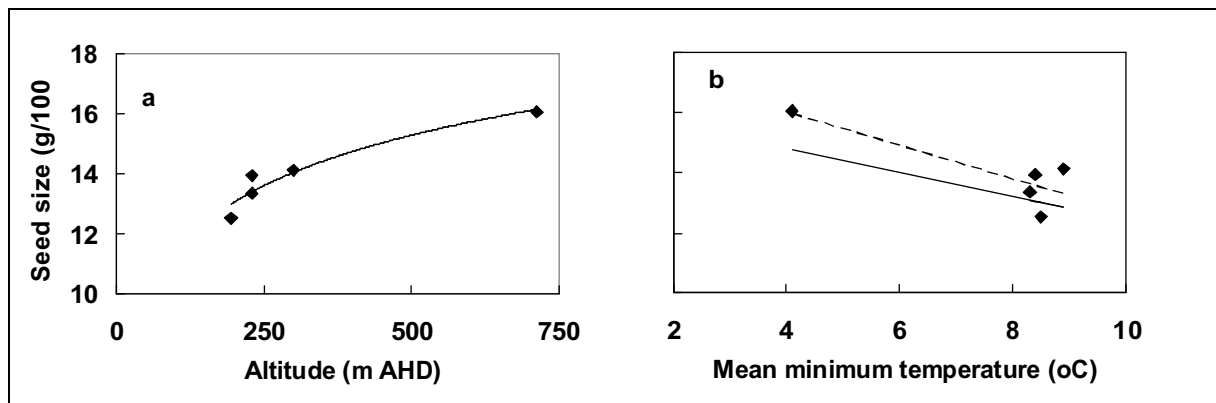


Figure 5 Effect of altitude (a) and temperature (b) on azuki seed size (g/100 seeds) for five grower sites, at a range of altitudes shown as Australian Height Datum (AHD), monitored during the summer of 1999/2000. The equation for the fitted curve (Fig 5a) is: Seed size (g/100) = 2.69 * ln(AHD) + 0.25 ($R^2=0.92$). Observed linear ($R^2=0.72$) (---) curve is compared with predicted linear curve (—) in Fig. 5b describing the relationship between seed size and mean minimum temperature during last three weeks of pod fill.

There was a distinct visual difference between azuki and kintoki seeds from each time of sowing in experiments conducted during the summers of 1997/98 and 1998/99. Azuki varied from dark red/maroon when planted early summer to a pale red/brown colour when planted late summer. The inverse was generally the case for kintoki. The relationship between L^* , a^* , and b^* values and Julian days at sowing is presented in Table 3. There was a highly significant ($P<0.01$) effect of time of sowing on these values.

Table 3 Effect of time of sowing (Julian days) on colour values for azuki and kintoki. L* brightness, a* redness, b* yellowness as measured by Minolta CR-310 colorimeter (50mm aperture) and H* hue and C* chroma as derived by equation 2.1

Treatment (sowing date)	Julian days	No.	L*	a*	b*	H*	C*
Azuki							
November 1997	337	2	30.34	16.02	6.03	20.6	17.1
December 1997	362	3	30.80	16.78	7.12	23.0	18.2
January 1998	390	3	32.65	17.20	8.81	27.1	19.3
February 1998	410	2	33.84	13.06	10.56	39.0	16.8
November 1998	318	3	37.96	12.54	3.65	16.2	13.1
December 1998	345	2	38.73	12.93	4.24	18.2	13.6
January 1999	374	3	39.26	14.48	5.43	20.6	15.5
February 1999	403	2	40.06	12.22	6.70	28.7	13.9
Kintoki							
November 1997	337	2	37.08	20.45	6.82	8.4	21.6
December 1997	362	3	30.82	19.98	4.25	12.0	20.4
January 1998	390	3	30.97	19.35	4.88	14.2	20.0
February 1998	410	2	28.88	16.16	3.06	10.7	16.4
November 1998	318	3	43.47	14.55	5.05	19.1	15.4
December 1998	345	2	40.26	13.50	4.00	16.5	14.1
January 1999	374	3	37.88	13.57	2.03	8.5	13.7
February 1999	403	2	38.66	14.94	2.18	8.3	15.1
5% l.s.d							
month x year x variety			1.21	1.60	0.85		
Significance			P<0.001	P=0.002	P<0.001		
Grower Sites (azuki 1999/2000)							
Bathurst	346		35.48	18.65	8.25	23.87	20.40
Canowindra	367		33.75	16.62	6.12	20.22	17.72
Eugowra 1	369		34.83	18.24	7.97	23.61	19.91
J.I.D.	378		35.21	18.32	8.21	24.15	20.08
Eugowra2	380		33.84	15.06	6.40	23.03	16.36

The best agreement was found between the mean minimum daily temperatures during the last three weeks of maturity and colour values for both azukis and kintokis. There was a very highly significant ($P<0.001$) interaction between variety, sowing date and year on L* and b* colour values and a highly significant ($P<0.01$) interaction on a* colour values (Table 3).

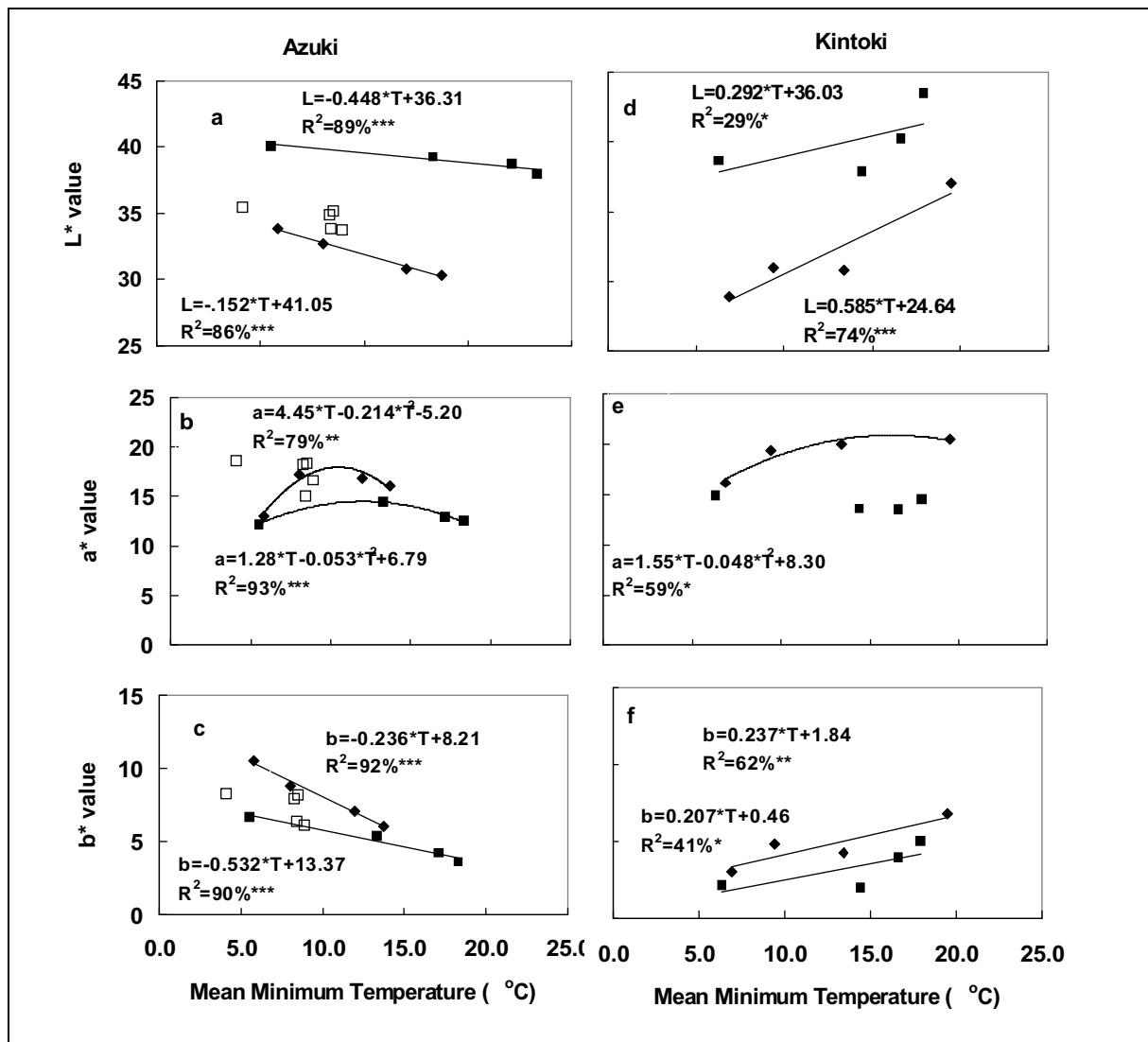


Figure 6. The correlation between mean minimum temperatures during pod fill and colour (L* a* b*) values for azuki (a,b,c) respectively and colour (L* a* b*) values for kintoki (d,e,f) respectively during 1997/98 (◆) and 1998/99 (■). Colour results from 5 commercial crops (□) grown in 1999/2000 are compared to experimental results. Significance of R² is indicated by *P<0.05, **P<0.01, *P<0.001.**

Of the environmental values that are likely to influence seed colour, minimum temperature during pod fill was strongly (P<0.001) negatively correlated (R² -0.94) with Julian days and best correlated with seed colour at maturity (Figure 6). Seed colour measurements from commercial azuki crops are also shown (Table 3) for comparison with measurements from field experiments. There was also good correlation between solar radiation and seed colour (data not shown).

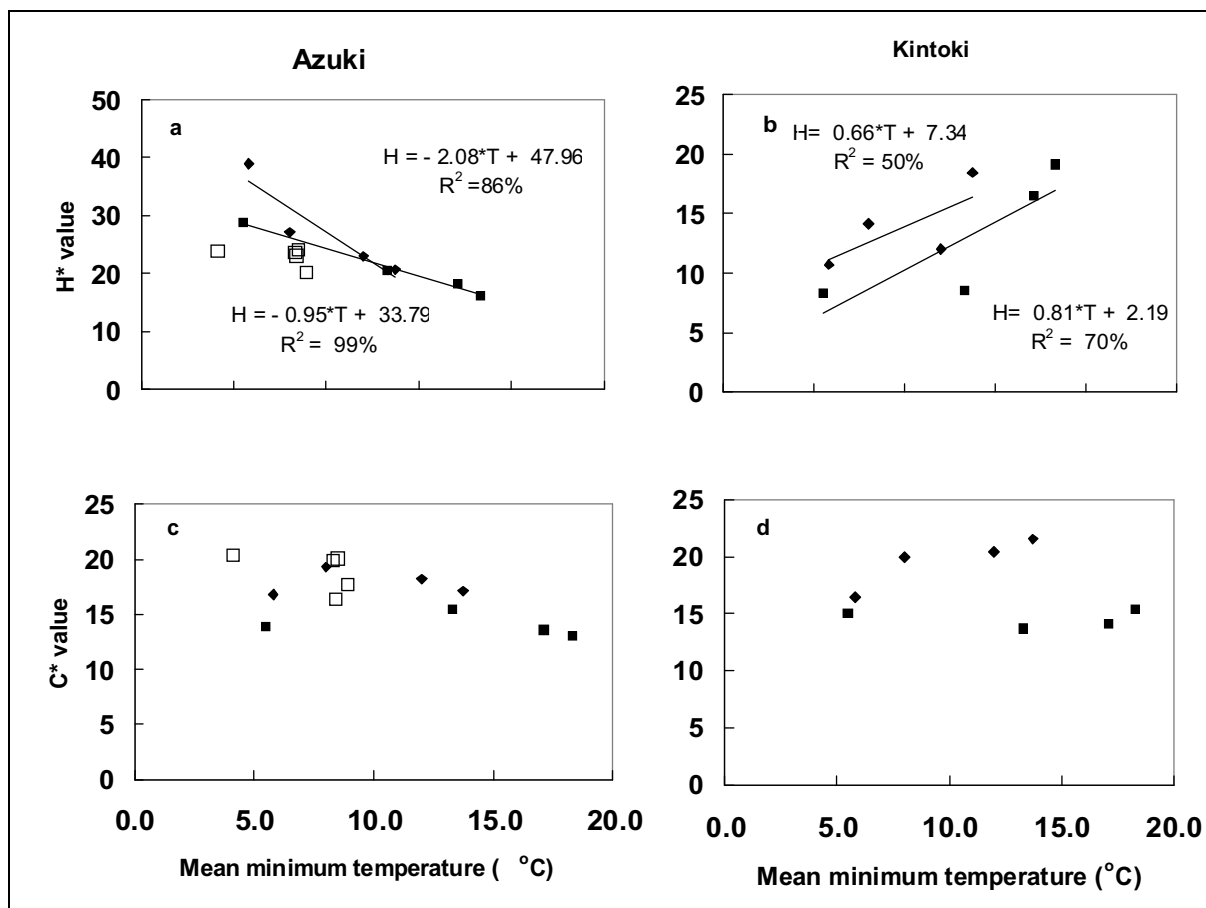


Figure 7 . The correlation between mean minimum temperatures during pod fill and Hue (H*) for azuki (a) and kin toki (b) and Chroma (C*) for azuki (c) and kintoki (d) during 1997/98 (◆) and 1998/99 (■). Colour results from 5 commercial azuki crops (□) grown in 1999/2000 are compared to experimental results.

Azuki showed highly significant ($P < 0.001$) negative linear correlations between minimum temperature and "L" and "b" values. However, the kintokis showed highly significant ($P < 0.001$) positive linear correlation between minimum temperature and "L" and "b" values. The correlation between the "a" value and minimum temperature was best described by quadratic functions in all cases except kintoki in 1998/99 where no correlation was found (Fig 6).

The relationship between hue angle (H*) and minimum temperature (Figure 7) was similar to that of the b* values for both azuki and kintoki. There was a negative linear relationship between H* and minimum temperature for azuki and a positive linear but weak ($R^2 = 0.50$ and 0.70) correlation for kintoki.

To determine whether solar radiation was directly influencing seed colour, measurements were made on two groups of seeds, one covered with silver foil and one left uncovered during seed development. Azuki pods covered in silver foil developed normally and both pods and seeds appeared to be similar to the control group which was not covered. There was no significant difference ($P > 0.05$) in colour values between covered and uncovered pods (Table 4). Thus solar radiation *per se* did not have a direct effect on L*a*b* colour values. As part of a wider study measurements were made using two different machines. There were very highly significant differences ($P < 0.001$) in colour values between the two machines used to record these values. However, there was no interaction between treatments and machines, and there was no effect of solar radiation on seed colour, regardless of the machine used to record these colour values.

Table 4 The effect of excluding solar radiation from maturing azuki seed pods on colour values: L* brightness, a* redness, b* yellowness as measured by Minolta CR-310 and CR-200 colorimeters and on H* hue and C* chroma.

Solar radiation (S)	Machine (M)	L*	a*	b*	H*	C*
Excluded	CR-310	34.43	17.43	7.17	22.37	18.87
Not excluded	CR-310	35.23	18.33	8.20	24.17	20.07
Excluded	CR-200	28.33	21.57	14.87	34.53	26.27
Not excluded	CR-200	30.00	23.27	14.30	31.60	27.30
Significance	Solar radiation	n.s.	n.s.	n.s.		
	Machine	P<0.001	P<0.001	P<0.001		

The within year effect of temperature on brightness (L*) was described by a linear regression (Figure 6). However, the reason for the difference between the 2 years is not apparent from this data. Tests on commercial samples during 2000 indicated that brightness was also influenced by the amount of dirt and dust on the seeds and by weather staining as a result of holes in pods caused by heliothis damage.

Japanese buyers rated azuki samples from these experiments for colour suitability. Seed samples rated as satisfactory by these buyers matured in environments where the mean minimum temperature during pod-fill was below 10°C. Preliminary reports indicate that kintoki beans need a similar (or perhaps slightly cooler) thermal environment to produce seed of a suitable colour.

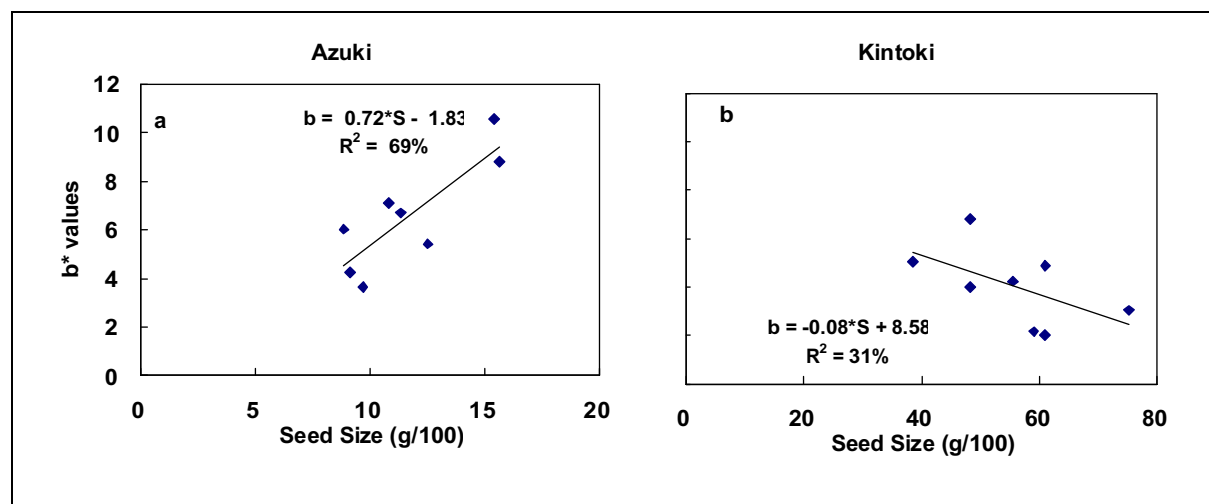


Figure 8 The correlation between seed size (g/100) and yellowness (b*) for azuki (a) and kintoki (b) at four times of sowing in two seasons.

There was a positive linear correlation for azuki ($R^2=0.69$) and a weak negative linear correlation ($R^2=0.31$) for kintoki between seed size and b* values (Figure 8). Azuki and kintoki 100 seed weight ranged from 9 to 16 g and 39 to 75g respectively, with corresponding b* values increasing from 4 to 11 for azuki and 2 to 7 for kintoki.

4. Discussion

These experiments have shown that the thermal environment during pod-fill influences the two most important aspects of azuki and kintoki seed quality, namely seed size and seed colour. Thus seed quality can be manipulated by choosing an appropriate sowing time for any given locality so that seeds mature during a relatively cooler period of the growing season. Altitude was shown to have an important influence on seed quality due to its impact on temperatures during the growing season.

Cooler air temperatures during seed maturity resulted in larger azuki and kintoki seeds. However, the effect of temperature on seed colour differs between azukis and kintokis. Cooler temperatures during pod-fill resulted in paler azukis and darker kintokis, of which both are generally preferred by Japanese importers.

Five commercial sites were chosen to obtain as large a spread in altitude as possible. However, each grower chose to sow at, what he considered to be, the ideal sowing time to achieve optimum azuki seed quality. This resulted in four of the five sites having similar thermal environments during the growing season. Thus, when investigating factors such as the effect of temperature on pod fill, four of the five data points were very similar and differed widely from the fifth (Bathurst crop). This made interpretation of these results difficult.

There is a clear relationship between seed size and the thermal environment during the pod filling stage of azukis. However, in this experiment, plant population density ranged from 34 to 48 plants per square metre, despite all crops sown at target densities of 40 to 50 plants per square metre. This may have contributed to seed size being larger than was predicted from previous experiments where plots were hand-thinned to 50 plants per square metre. This demonstrates the challenges faced when extrapolating results from small plot experiments to a commercial situation.

The maximum azuki above ground biomass for the five sites averaged 805g/m² which was 12% higher than the average of 710g/m² for November, December and January sowings in a small plot experiment conducted at the J.I.D. site during 1998/99. However, the peak biomass for azuki samples from the commercial crop sown in January 2000 at the J.I.D. site was 650g/m² which is similar to the 620g/m² obtained from azuki sown in January at J.I.D. in small plot experiment. Thus yields obtained in small plot experiments were representative of those obtained in commercial azuki crops grown over a wider climatic range.

Yield components measured in crops in commercial fields differed from those measured in previous experiments. This could be due to plant population effects. The two crops with the least branches per plant (1.7 and 1.8) had the highest two population densities of 41 and 48 plants/m², respectively. This is in contrast to the crop with the highest number of branches (1.7/plant) and lowest density (34plants/m²). Thus the effect of plant population needs to be considered when interpreting data about azuki yield components.

Most of the effect of altitude on seed size can be explained in terms of the direct impact altitude (at common latitudes) has on the thermal environment when these five crops matured at similar times in autumn. Other factors such as crop nutrition and plant population may account for the agreement ($R^2=0.92$) between altitude (ie location) and seed size being better than the correlation ($R^2=0.72$) between thermal environment during pod-fill and seed size. Further experiments aimed at investigating these factors, using a larger data set may be possible with azuki growers (2000/2001 season) now located from Rylestone in the central tablelands of NSW to Narrandera in the South West Plains of NSW.

Colour measurements from Field Experiments

The results of this research indicate a strong correlation between time of sowing and seed colour. For azuki, early sowing produced dark red beans whereas pale red seeds were produced with later sowing. The converse is true for kintoki. Seed testa colour was formed during the last few weeks of maturity. Lower temperatures increased for azukis (and decreased for kintokis) the brightness (L^*) and yellowness (b^*) and hue (H^*) of azuki, without greatly effecting the redness (a^*) or chroma (C^*) of either species. It is hypothesised that cooler conditions during pod fill and maturation (due to late sowing or altitude) will produce brighter, paler azuki and darker kintoki, both of which are desired by Japanese importers. Kato *et al* (2000) have also shown a positive correlation between L^* and minimum temperature during ripening of azuki.

Standard protocols, quantifying seed colour, would facilitate "sale by description" and contract growing of azuki. For example "No.1 Grade azuki" could be classified as having colour values defined as $L > 30$ and $b > 8$ (measured with a Minolta CR-310 50mm aperture) as suggested by Redden and Desborough (1996). Thus azuki sown only in January and February 1998 would have been classified as "No 1 Grade azuki".

Kato *et al* (2000) found that seed from different sources could be discriminated on the basis of two dimensional colour values using L^* (brightness) and C^* (chroma) axes or L^* (brightness) and H^* (hue) axes. They reported that variation in L^* was greater than C^* between different growers, locations or years. These researchers also found variation in C^* greater between different azuki varieties than other factors. They also noted a strong ($R^2=0.82^{**}$) linear relationship between L^* and H^* values, and concluded that both these values were effected by weather conditions during maturing period of azuki regardless of variety. An attempt to use this two-dimensional mapping of L^* and H^* or L^* and C^* values using data from the five grower sites in the current experiment proved unsatisfactory.

Although quadratic regressions were fitted to the a^* values obtained in previous experiments (Figure 6), it is suspected that this is due to single outlying points at the start or end of the season resulting in these quadratic curves being significant. Thus there may be no underlying relationship between temperature and redness (a^* values). If frosting influenced the colour of seed from crops sown in February and these are not included in the analysis then there is a linear relationship between a^* values and minimum temperature during. Further work using a larger data set would verify whether this is the case.

There was insufficient data to form conclusions about the effect of severity of insect damage on seed colour and brightness. Surface contamination of seeds with dust particles at harvest has also been cited by growers as a possible cause of low L^* values. Seed polishing machines are used in Japan prior to resale to end-users. Further tests comparing colour values of polished and unpolished seeds would confirm whether azuki growers should polish seed before testing and sale.

Climate modelling work could also help identify regions suitable to growing premium azuki and kintoki and identify the optimum planting time for each region. It would be more practical to use temperature data rather than solar radiation for these models due to its wider availability.

Further research about the biochemical mechanisms of seed testa colour development in both azuki and kintoki would help understand the influence of environmental factors such as minimum temperature on these processes. It may also resolve why cooler temperatures during pod-fill make azuki seed paler but make kintoki seeds darker in testa colour. For example, in studies on common beans, Gantet *et al* (1993 and 1993a) have examined the genetic and environmental factors controlling anthocyanin accumulation in bean pods and pulvini. These studies found that a developmental switch is a prerequisite for anthocyanin accumulation in the pods. This does not occur before day 4 after

pollination and is controlled by light in competent pods. This supports the rationale for relating environmental conditions experienced after pod initiation, to seed colour. However, temperature rather than light appears to be determining seed testa colour in azuki and kintoki.

Various researchers (Curry 1997, Arakawa 1991 and Faragher 1983) have investigated the effect of light and temperature on anthocyanin accumulation in apple skin. They found that different cultivars responded differently to pre cooling then incubation at various temperatures. The main determinant for anthocyanin production in the fruit skin is thought to be the amount of phytochrome present. Phytochrome metabolism is highly governed by temperature. Arakawa found that in 'Jonathan' apples the optimum temperature for anthocyanin accumulation increased from 15°C to 25°C in non bagged fruit and from 20°C to 25°C in bagged fruit as it ripened. It would appear, from research reported here, that temperature also governs aspects of seed colour in azuki and kintoki.

There is a positive linear relationship between seed size and minimum temperature during pod-fill and between L* and b* and minimum temperatures. Thus it was hypothesised that seed size may be closely related to L* and b* values and that these values could be estimated indirectly by measuring seed size. There was no agreement between L* values and seed size in the current experiments, but there was a positive linear correlation for azuki ($R^2=0.69$) and a weak negative linear correlation ($R^2=0.31$) for kintoki, between seed size and b* values. This correlation may not be sufficient to reliably estimate seed colour from seed size.

It has been suggested (Desborough pers.comm.) that the thickness of azuki seed testa may influence seed colour. Thickness may be related to seed size, in that as seed size increases, the seed testa may become thinner and colour within this layer may become more dilute. Further research would verify whether this is the case, and determine whether temperature during pod-fill has a direct effect on seed testa thickness.

Comparison between Colorimeters and methodology

The problems of not having a standard protocol to compare objective colour tests are demonstrated in this section. There is a significant effect of machine (or usage of the machine, ie hilums facing the light source or orientated randomly) on all colour values. The machine with the larger aperture (the CR-310) would be more suited to rapid seed analysis. The 50mm aperture can read a larger sample rather than having to perform many repeat tests on a single sample using the CR-200 with an 8mm aperture which can only read 2, 3 or possibly 4 seeds at a time. The practice of turning white hilums away from the measuring source also appears to be impractical. It is unlikely that measurements made in Australia using a Minolta CR-310 (50mm aperture) could be reliably compared to measurements made elsewhere with a Minolta CR-200 (8mm aperture) or similar machine.

One group of researchers (Kato *et al* 2000) have documented their measurement techniques and use a Tokyo Denshoku TC-1800 Mkii Colour Analyser (Tokyo Denshoku Corp. Tokyo Japan). Reference samples of azuki seed should be jointly analysed with this machine and the Minolta CR-200 (8mm aperture) to see if they give comparable readings or at least establish whether one set of readings could be correlated to the other set. Alternatively, growers could insist samples are analysed in Japan using a Minolta CR-310 which has a large (50mm) aperture, suited to reliable, rapid seed colour analysis.

5.Implications

The research described in this report has shown that delaying planting is an effective strategy to improve seed quality. However, delayed planting also results in decreased seed yield. Thus a trade-off must be found between planting early for yield and late for quality. Seed quality decision models may assist making this choice.

This research has already helped growers fine-tune their planting date, and has given planting date guidelines to prospective kintoki growers. An initial seed quality model, which will predict seed colour and size for various planting dates at a specified location, will have wider applicability.

6.Recommendations

This work will shortly be published in a thesis, which will be available at The University of Sydney Library. The major recommendations arising from this report are:

1. Azuki and kintoki planting should be delayed as late as practical to improve seed quality. This usually involves a compromise between seed quality and seed yield.
2. This delayed planting should be targeted so that azuki and kintoki pods will mature when the daily mean minimum temperature is below 10°C.
3. A seed quality decision support model, developed as part of this thesis, should be refined and made available to growers and advisers to aid in selecting the appropriate planting date for a chosen locality.
4. Key findings from this report should be incorporated into the next NSW Agriculture Azuki Agfact.
5. An ongoing assessment of other factors influencing seed quality such as nitrogen application, the impact of dust and insect damage and hard seeds should be made.
6. The impact of polishing seed on colour and brightness should be examined.
7. An agreed machine and protocol for testing seed colour should be negotiated with Japanese importers so that disputes could be referred to an independent arbiter.

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