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Edamame Soybean Development in Australia

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Foreword

Edamame is soybean which is harvested as a vegetable when the seedpods are at their largest but before any yellowing has occurred. The key determinants of quality in edamame are large seed size, high sugar content and bright green colour.

The work in this project sought to understand the ripening process in Australian–bred and elite Asian cultivars of edamame soybean, and in so doing understand how changes in seed size and sugar content of the seed vary with the ripening, cultivar, agronomy and environment. The project showed that in well-watered plants, sugars accumulate at about the same rate as seed expansion. Seeds are largest and sugar content of seed highest in crops grown at low plant population and maintained in well watered condition. Edamame should be harvested when seed size is maximised but before any yellowing of the pods occur. An indicator that this is about to happen is that the lower leaves on the plant start to yellow. Alternately a more accurate measure is that the rate of increase in fresh weight of seed has slowed or maximised indicating that physiological maturity has been reached and therefore quality for vegetable use has also peaked and started to decline. Edamame grown under these conditions from the Japanese cultivar, Tanbaguro, the Taiwanese cultivar KS#1 and the Australian cultivars C784 and Bunya were acceptable to consumers in both fresh and frozen form.

The project also established the limits of potential adaptation for all four cultivars evaluated. The Asian cultivars were generally poorly adapted due to narrow planting window, uneven ripening, difficulty of seed production and susceptibility to several diseases. The cultivar C784 was discovered to be highly susceptible to *Phytophthora* root rot during the course of the studies, but the cultivar Bunya warrants commercial evaluation. Plentiful supplies of Bunya seed are available. The cultivar is broadly adapted to a wide planting window over the summer period from around Sydney, NSW north to the Atherton Tablelands in Qld. In warmer environments such as Bowen and the Burdekin region of Qld, Bunya is potentially adapted year-round. Commercial development should focus on development of grower skills in maximising quality particularly in the areas of insect control, harvest timing and post harvest handling.

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Peter O'Brien
Managing Director
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Acknowledgments

The assistance of Mr Adam Willson in the conduct of commercial scale trials is gratefully acknowledged.

Contents

Executive Summary

What is the report about?
This report sought to provide underpinning of the development of an Edamame (vegetable soybean) industry in Australia via a critical understanding of the ripening process in CSIRO-Bred and elite Japanese varieties of edamame, and in particular understand how changes in seed size and in the seed content of sugars vary with ripening or environment.

Who is the report targeted at?
People seeking to establish a commercial industry should find the information contained in this report useful. In particular this report establishes a baseline for the agronomics necessary to produce good quality edamame. Growers and advisors may then optimise the production environment with an understanding of the likely effect of different agronomic methods.

Background
Edamame is a flavoursome and healthy vegetable than can be fun to eat. The traditional setting for consumption is as a snack consumed with beer in a commercial establishment.

Edamame is a vegetable soybean which is harvested when the seedpods are at their largest but before any yellowing has occurred. Production of the crop in Australia has been small and beset with difficulties associated with seed supply, poorly adapted varieties and variable quality. It is therefore a relatively unexploited new vegetable crop for Australia.

Aims and Objectives
The research reported here sought to underpin the potential development of a commercial edamame industry in Australia via an improved understanding of the key determinants of quality and means to influence this quality.

Methods used
A series of experiments were conducted to establish the effect of agronomic variables such as water stress, cultivar, plant population, thinning and window of adaptation on the potential for production and likely effect on quality. In addition some field scale evaluation and consumer acceptance was tested.

Results and Key findings
The project found that the key determinants of quality in edamame are large seed size, high sugar content and bright green colour. The project showed that in well-watered plants, sugars accumulate at about the same rate as seed expansion. Seeds are largest and sugar content of seed highest in crops grown at low plant population and maintained in well watered condition. Edamame should be harvested when seed size is maximised but before any yellowing of the pods occur. An indicator that this is about to happen is that the lower leaves on the plant start to yellow. Preliminary market testing has shown that fresh beans can be held in excellent condition for several days using technology similar to that deployed for some other vegetables. The edamame can be transported to distant markets during this time.

Edamame grown during this project from the Japanese cultivar, Tanbaguro, the Taiwanese cultivar KS#1 and the Australian cultivars C784 and Bunya were acceptable to consumers in both fresh and frozen form.

The project also established the limits of potential adaptation for all four cultivars evaluated. The Asian cultivars were generally poorly adapted due to narrow planting window, uneven ripening, difficulty of seed production and susceptibility to several diseases. The cultivar C784 was discovered to be highly susceptible to *Phytophthora* root rot during the course of the studies, but the cultivar Bunya shows potential for commercial evaluation. Plentiful supplies of Bunya seed are available. The cultivar is broadly adapted to a wide planting window over the summer period from around Sydney, NSW north to the Atherton Tablelands in Qld. In warmer environments such as Bowen and the Burdekin region of Qld, Bunya is potentially adapted year-round. Commercial development should focus on development of grower skills in maximising quality particularly in the areas of insect control, harvest timing and post harvest handling.

Implications for relevant stakeholders.
This project has shown that good quality edamame can indeed be produced in Australia via the use of an adapted cultivar and good agronomic conditions, and harvest at optimum time. The edamame thus produced appears to meet with ready acceptance from the market.

Recommendations
1. Edamame should be gown as a vegetable crop under highly managed conditions.
2. The variety Bunya appears adequate to commence commercial development of the industry, however varieties with larger seed size and possibly also with improved sensory attributes would be desirable.
3. At harvest time, care needs to be taken to ensure that seeds are large and bright green in colour. If harvested at this stage of development, the vegetable appears robust enough to undergo freezing or transport under chilled conditions to distant markets. It also appears that there may be opportunities to develop supply relationships with specific high-end restaurants.

1. Introduction

Edamame is a traditional food of Japan and China that is now consumed throughout east-Asia (Yinbo et al., 1997, Cui et al. 2004). Traditionally, the whole plant is harvested green at the R6 or R7 stage (Fehr et al., 1971; Mebrahtu et al., 1991; Rao et al., 2002) and transported intact to market to assure customers of the freshness of the product. After purchase, pods are removed from the plant, boiled and consumed as a snack food. In Japan, the common use for edamame is as a snack consumed with beer in commercial establishments. Boiled salted pods should be blemish-free and bright green (Shanmugasundarum et al., 1997; Carter and Shanmugasundarum, 1993). Traditionally, cultivars with green seed coat and cotyledon at maturity have been preferred by growers because the harvest period can be extended closer to maturity of the plant without experiencing the yellowing associated with maturity. Seed pods should have sparse gray pubescence and contain three seeds per pod, though two seeded pods are acceptable in the market (Konovsky et al., 1994). There should be an absolute minimum of one seeded pods because they are disliked by the consumer, requiring greater effort to shell. Four seeds in a pod are not preferred because the number four is considered unlucky in Japanese culture. In recent times a reselection of the old Japanese cultivar Tanbaguro has become popular for edamame because of its exceptionally smooth texture, high sugar content, large seed and good flavour in spite of it having a black seed coat and stiff tawny pubescence on the pod.

Desirable edamame should have very large seed, high sugar levels, and a smooth texture (Konovsky et al., 1994). Varieties suitable for edamame generally possess greater than 10% dry weight of sucrose from mid pod development until maturity (Masuda and Harada 2000). It is thought that the genetic removal of lipoxygenases will result in a bean with less beany flavor and greater acceptability to the market (Shanmugasundarum et al., 1997). Young et al. (2000) found that beans that were sweet were also somewhat nutty, less beany flavored, slightly oily, without an unpleasant aftertaste, and generally better in overall eating quality. This is not surprising given that sugar content is positively correlated with oil content and negatively with protein (Krober and Cartter, 1962; Geater et al., 2000; Hartwig et al., 1997; Wilcox and Shibles, 2001). For the fresh-frozen market, uniformity of maturity, a thicker pod wall to reduce freezing damage and plant habit to permit mechanized harvest is required in addition to the quality traits required in the fresh product (Chotiyarnwong et al., 1996). Cultivar development for edamame for the fresh market should focus on production in multiple sequential planting dates so that the harvest period can be maximized.

In Australia, work on development of edamame has occurred at Yanco, Gosford, Grafton and Gatton on and off under the auspice of state departments (Nguyen 1998, James 2004, James 2005). This research has generally used imported varieties with generally poor adaptation to the photo-thermal regime found in Australian vegetable growing regions, or if Australian-adapted material was used it did not meet necessary quality requirements.

Modern markets for edamame pay the highest premium for freshly harvested beans presented in attached form. This product receives a substantial premium over the price of frozen beans. An opportunity exists for the production of edamame to supply the Australian market all year round through the use of staggered sowing dates and production areas spread over a wide range of latitudes. Recent changes to the quarantine restriction for import of edamame into Japan may also mean that fresh attached beans may be able to be exported to Japan during the northern hemisphere off season, a time in which fresh edamame currently receives a three to five fold premium over the frozen product.

Part of the desirability of introduction of edamame for the Australian consumer stems from the widely-reported health benefits from inclusion of soybean into western diets. All soybean contains isoflavones which act as anti-oxidants and as weak oestrogens which appear to reduce risk of certain cancers and of coronary artery disease. Edamame is more palatable to western consumers than other soy products and may therefore more easily permit inclusion of soy into the diet.

Experimental plan

This project sought to determine means to assure quality of Australian-grown edamame. A series of experiments was therefore conducted to determine the relative importance of agronomic treatments such as water stress, choice of cultivar, plant population, planting date and location. These experiments were followed up by field scale testing in farmers' fields, test freezing and by general evaluation for acceptance by consumers in farmers markets and restaurants.

Effects of water stress on seed size and sugar content

Methods

Experiments were conducted in the rain-out shelter and adjacent field at the CSIRO Cooper field station at Gatton in southern Qld. Five Australian bred varieties were grown at a plant population of 20 plants per m^2 under either terminal water deficit or watered after each 60 mm of cumulative pan evaporation. The final irrigation in the terminal water stress treatment occurred at 70 days after sowing, which was about 25 days after flowering. The experiment was replicated twice.

Plants were harvested and pods removed at six intervals during pod development. Seeds were dissected from pods, fresh and dry weight recorded and seed assayed for sugar content (sucrose, glucose, raffinose and stachyose).

Results and Discussion

Both seed size (Figure 1) and sugar content (Figure 2) were adversely affected by water stress. At the time approaching harvest maturity for edamame, moisture content of fresh seed was around 50 to 60 percent. Seed size was reduced to below 30 grams per 100 seed dry weight which is a suggested minimum standard for edamame and sugar content to below 10 percent dry weight, also a suggested minimum standard. Data is presented averaged across cultivars within a harvest date because there were no significant differences ($P < 0.05$) between cultivars in this study.

Figure 1. Seed size, averaged over five varieties of edamame soybean grown under well watered conditions or terminal water deficit. Treatments are significantly different ($P < 0.05$) for the later five harvests.

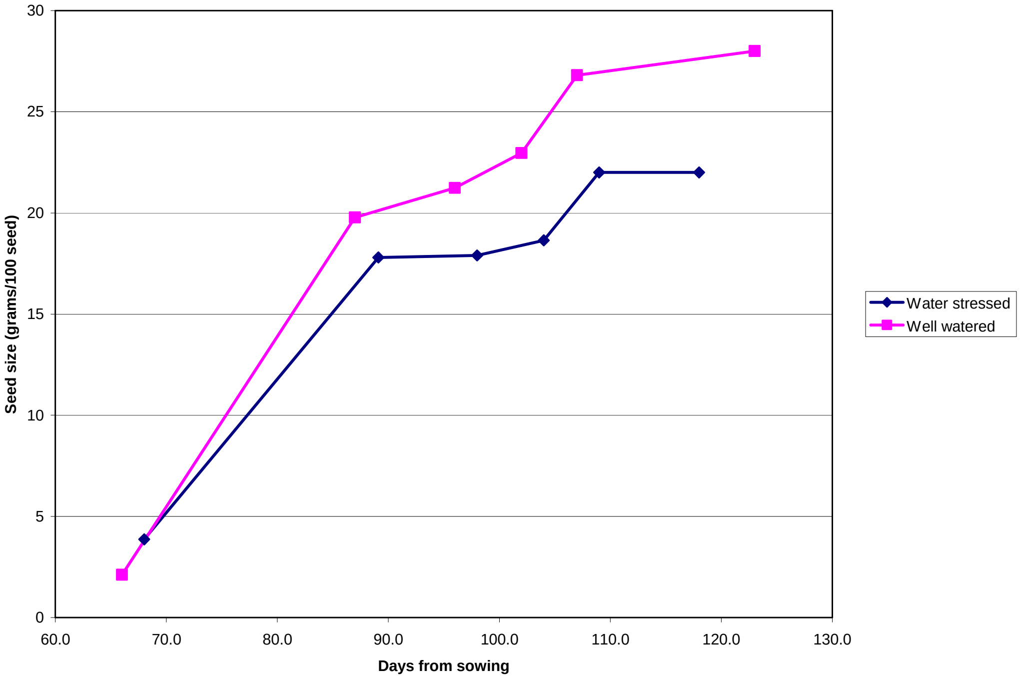
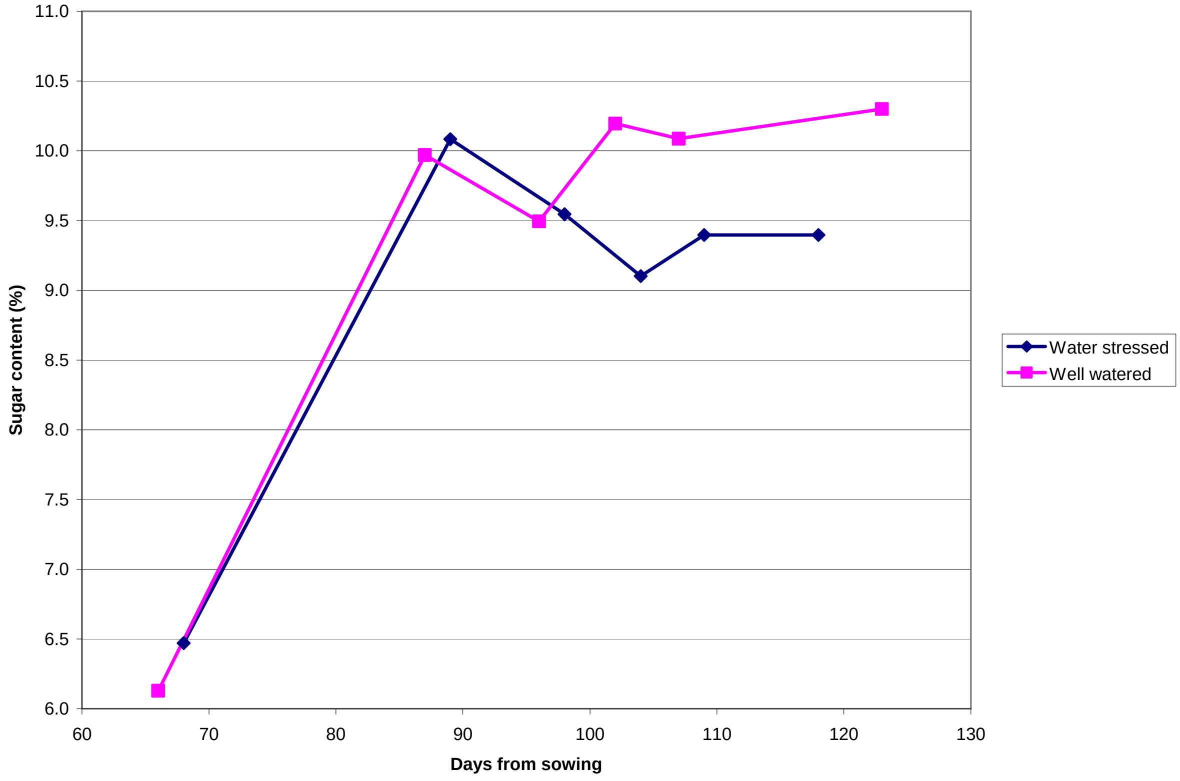


Figure 2. Total sugar content (dry matter basis), averaged over five varieties of edamame soybean grown under well watered conditions or terminal water deficit. Treatments are significantly different (P <0.05) for the later three harvests.



Effect of cultivar on sugar content and seed size of edamame soybean.

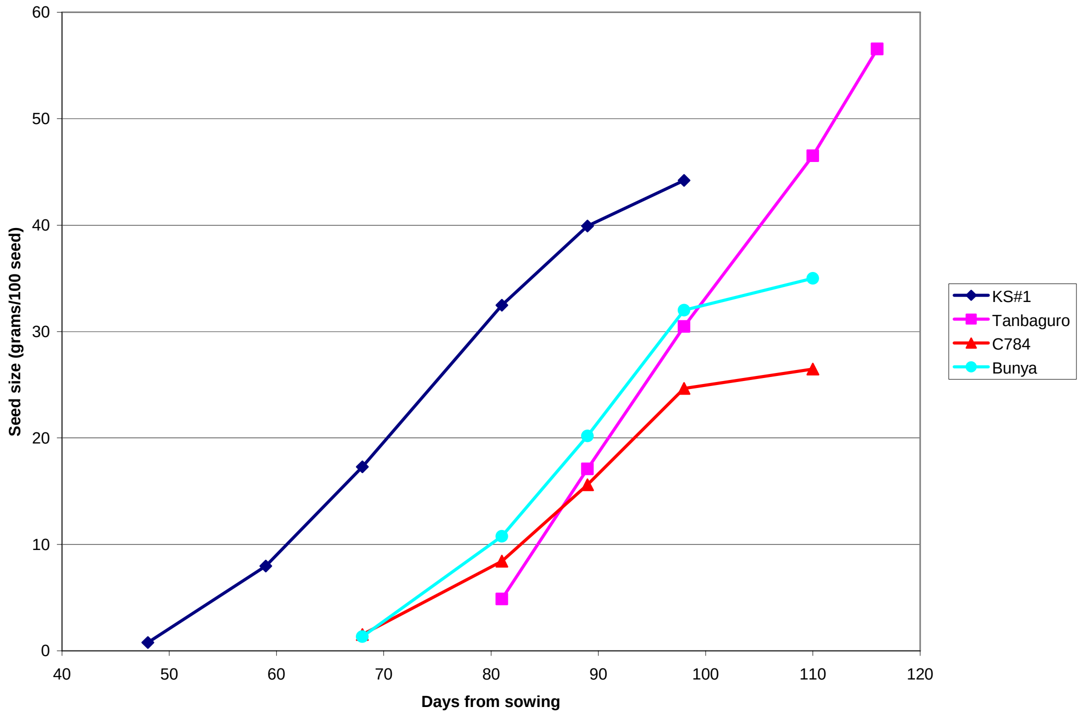
Methods

A trial was conducted at Lowood in Southern Qld to evaluate content of sugars and seed size of four cultivars of edamame soybean, replicated three times. The trial was maintained in well watered condition by watering after each 60 mm of cumulative pan evaporation, but relatively severe damage to the trial occurred due to a severe outbreak of a multi-insecticide resistant strain of whitefly.

Results and Discussion

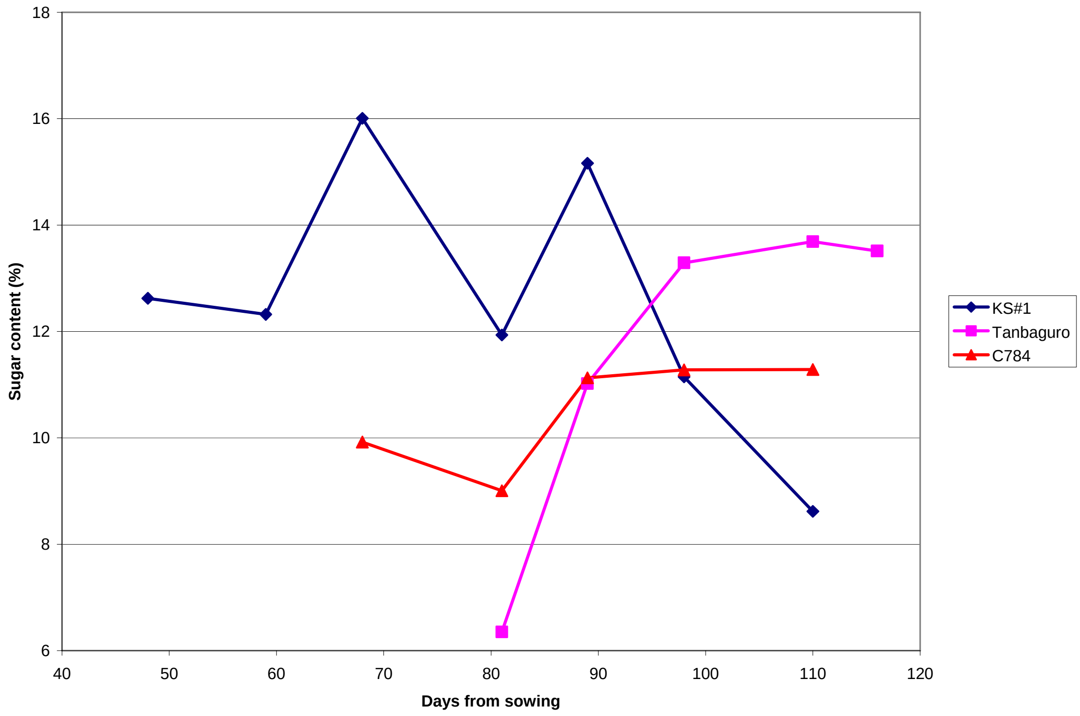
Cultivars differed in the time of commencement of pod fill due to differing time of flowering. However, once pod fill had commenced, seed size increased linearly almost until the time of harvest maturity (Figure 3). Maximum seed size was greatest for the premium Japanese cultivar Tanbaguro with 110 g fresh or 58 g dry /100 seed, followed by KS#1 at 80 and 44, Bunya at 75 and 36 and C784 at 58 and 28 g/100 seed respectively.

Figure 3. Seed size (dry weight) from 20 days after flowering until harvest maturity for four varieties of edamame soybean grown at Lowood in southern Qld. Size of harvest-mature seeds were significantly different for each cultivar (P <0.05).



Total content of sugars was only assayed for KS#1, Tanbaguro and C784 (Figure 4) as at the time of the experimentation, the variety Bunya was not under consideration for use as edamame. Brix of crushed seed was measured using a hand-held instrument. However, no differences in brix between cultivars were able to be detected and the brix meter gave rather indistinct results. Sugar content was highly variable across harvest dates and between cultivars possibly due to a confounding effect caused by a severe outbreak of the sap sucking insect whitefly.   At harvest maturity, differences in sugar content between cultivars was not statistically significant, however the sugar content of KS#1 fell from earlier harvested samples, possibly due to the extreme sensitivity of this cultivar to whitefly attack.

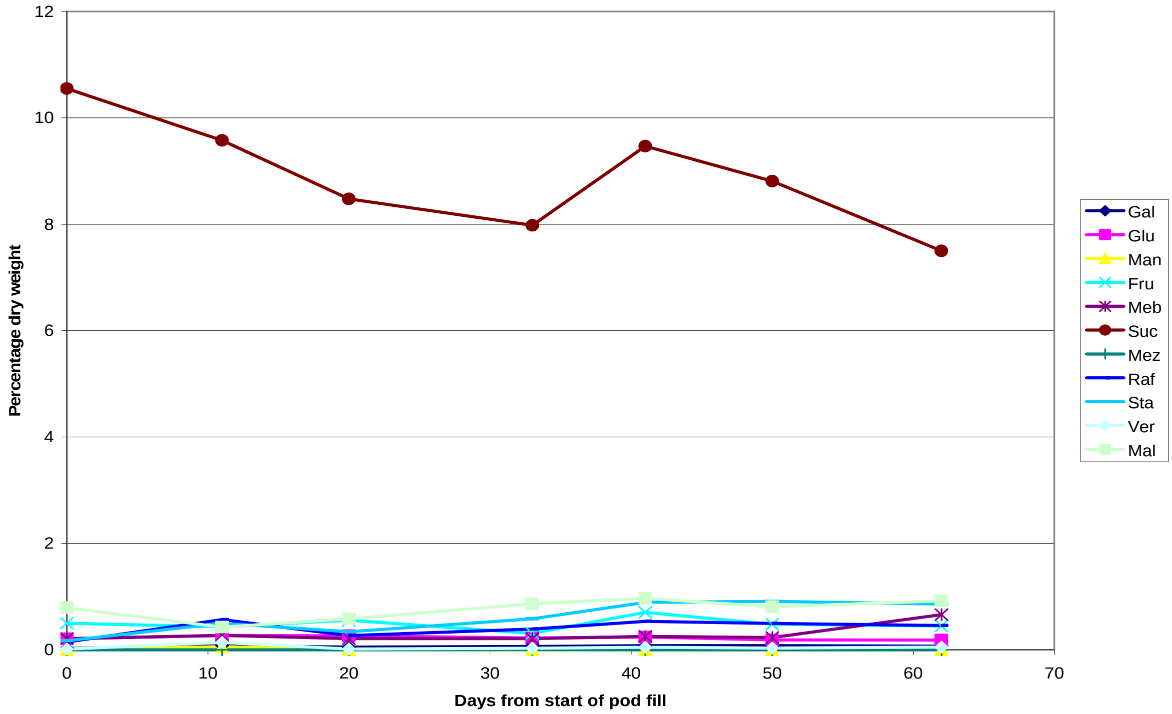
Figure 4. Total sugar content (dry matter basis) from 20 days after flowering until harvest maturity for three varieties of edamame soybean grown at Lowood in southern Qld. Sugar content of cultivars from day 90 onward was not significantly different (P <0.05).

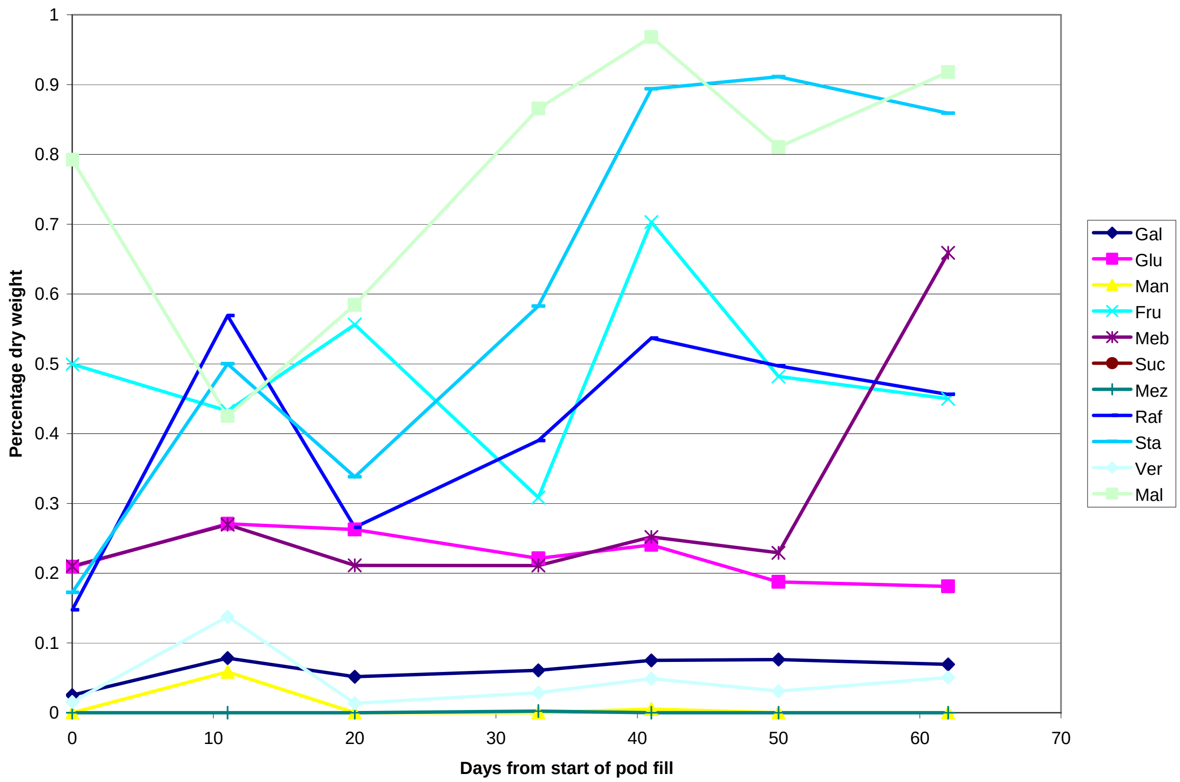


The type of sugar present was assayed separately, but is presented here averaged over the three cultivars (Figure 5) due to differences between the cultivars being not significantly (P < 0.05) different from one another. Dry matter content of sugars was generally consistent over time with Sucrose being the principal sugar present at about nine percent dry weight. The next highest contents of sugars were maltose and stachyose at about one percent and fructose and raffinose at about half a percent dry weight.

Sucrose, glucose, fructose and maltose and to a lessor extent stachyose and raffinose are the principal sugars associated with palatability. All appear to accumulate at about the same rate as the seed increases in size. Apart from instances where interrupted development occurs, such as drought stress or whitefly attack, it is clear that there is no particular point in pod development where sugars peak or are maximised. It is therefore suggested that seed size and colour are likely to be more useful as indicators of harvest maturity than measures of sugar content.

Figure 5a (top) Content of sugars (dry matter basis) from the start of podfill until harvest maturity averaged over three varieties of edamame soybean grown at Lowood in southern Qld. Content of galactose (gal), glucose (glu), mannose (man), fructose (fru), melibiose (meb), sucrose (suc), melezitose (mez), raffinise (raf), stachiose (sta), verbascose, (ver) and maltose (mal) is shown over time.
Figure 5b (bottom) Content of sugars with the 0 to 1 percent dry weight range scaled up.





Effect of plant population on seed size of edamame soybean.

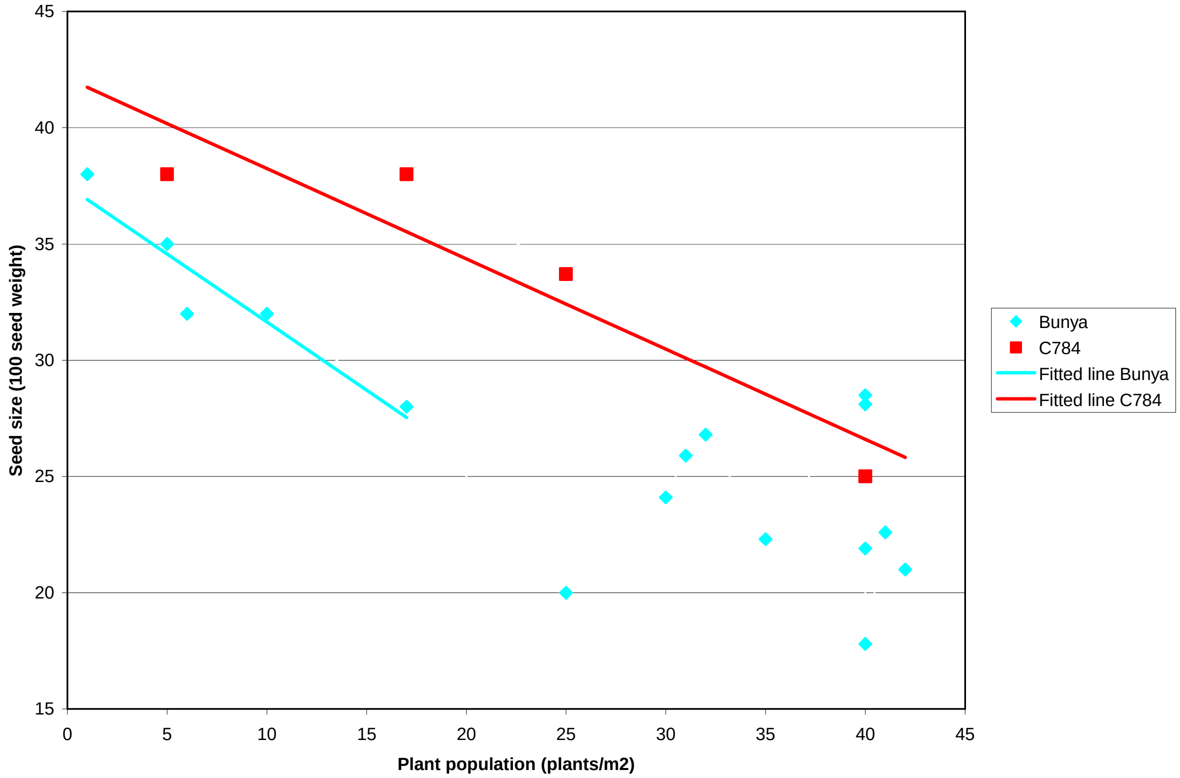
Methods

Plant population and seed size of the cultivars Bunya and C784 was recorded in varietal evaluation trials and specific plant population studies conducted across southern and north Qld (Experiment 1). Another experiment conducted at Walkamin on the Atherton Tablelands in north Qld evaluated the effect of thinning at flowering from 30 pl/m^2 to plant populations of 5, 10 and 15 pl/m^2 (Experiment 2).

Results and discussion

Experiment 1. Seed size of both cultivars increased linearly as plant population decreased (Figure 6). Although if a restricted data set of > 20 pl/m2 is used for the cultivar Bunya, as would be normal for grain-growing environments, over the 20 to 40 pl/m^2 range there is no effect of plant population on seed size.

Figure 6. Seed size of edamame varieties Bunya and C784 when grown at plant populations ranging from 40 plants/m^2 down to around 5 plants/m^2.



Experiment 2.
There was no effect of thinning at flowering on seed size for C784 (Table 1). It is therefore suggested that in order to maximise potential seed size and therefore quality of edamame, a plant population below 10 pl/m^2 needs to be established.

Table 1. Effect of thinning at flowering on dry weight of mature seeds of the edamame variety C784. Differences are not statistically significant (P. < 0.05).

Plants/m^2	5	10	15	20
Seed size (grams/100 seed)	28.2	32.5	26.7	28.5

Adaptation to planting window and regional adaptation of cultivars.

Introduction

Flowering is an important stage in the development of a crop. The timing of flowering also tends to be the major determinate of potential vegetative biomass and therefore yield and potential adaptation in edamame soybean, since this is the time that vegetative growth ceases altogether in most varieties. Flowering is also a time when the effects of environment, such as heat or drought stress can have an especially large negative effect on pod set. It is therefore important to try to have flowering correspond with relatively mild environmental conditions.   The rate of development model (RoDMod) was developed to permit quantitative analysis of the effect of photoperiod and temperature on a given genotype of soybean (Summerfield *et al*. 1991). It is therefore a powerful tool to help understand the potential range of adaptation of edamame soybean to different environments.

Key to the understanding of the flowering response of soybean to daylength within the agronomic range of environments is the concept of critical daylength $P_{c.}$ This is the daylength where the flowering response changes from one driven by temperature alone when daylengths are shorter than the P_c and driven by a combination of daylength and temperature when daylengths are longer than the P_c. In effect this means that long days, such as in summer, tend to delay flowering. On the other hand warm temperatures tend to promote flowering. Each variety of soybean has a very specific response to daylength and temperature. It is for this reason that most soybean varieties tend to be adapted to a specific region and are poorly adapted to other regions. Time of flowering is relatively simple to predict from daylength and temperature data for a given region. The prediction can be used to assess which cultivars might reasonably be adapted to a particular region and to particular sowing dates within a region.

Materials

The trial areas were prepared as for normal procedures at the site. Trials were sown in a randomised complete block design with two replicates. Within each replicate, varieties were sown in single-row plots each 1 m in length, spaced at 1 m apart and sown with sufficient seed to establish 10 plants per metre of row. Phenology was recorded for each plot and weather data consisting of maximum and minimum daily temperatures and rainfall was collected at a nearby weather station.

The controlled environment facility trials were conducted in the controlled environment facility of the CSIRO at the Qld Bioscience Precinct in Brisbane. Two replicates were sown within a temperature and daylength treatment. Each replicate consisted of a single pot with four plants. Pots were placed in trays filled to a depth of 2cm with water. Phenology was recorded for each pot, daylength and temperature data was logged using the computerised recording system of the facility.

Trials were conducted at the locations shown in Table 2 and varieties used in the modelling of time to flowering are listed in Table 3.

Table 2. Locations and seasons in which data for phenology and weather conditions were recorded.			
	Spring	Summer	Winter
Atherton, north Qld	●	●	●
Ayr, north Qld	●	●	●
Townsville, Australia		●	●
Gatton, Australia		●	
Controlled environment Australia	16 hour days with mean temperature 30^{o}C 14 hour days with mean temperature 18^{o}C 10 hour days with mean temperature 20^{o}C 10 hour days with mean temperature 30^{o}C		

Table 3. Varieties of edamame soybean under evaluation for adaptation to daylengths and temperatures of potential cropping environments.	
Variety	Origin
KS#1	Japan via Taiwan
Tanbaguro	Japan
C784	Australia
Bunya	Australia

Results

Time to first open flower varies from site to site and for different varieties. Fastest time to flowering always occurred with the variety KS#1. The other three varieties re-ranked in time to flower at different sites and different planting dates. The flowering data was combined with temperature and daylength records and the flowering response modelled using RoDMod (Summerfield *et al*. 1991). The regression equations thus calculated are listed in Table 4.

Table 3. Regression equations to estimate the rate of development toward flowering (R) from mean pre-flowering temperature (T) and mean pre-flowering daylength (P).			
	$P < P_c$	$P > P_c$	R^2
KS #1	R = 0.00548+0.00107*T	R=0.1436+(-0.000098*T) + (0.00775*P)	86***
Tanbaguro	R = 0.00845+0.00103*T	R=0.0133+(0.001952*T) - (0.00288*P)	94***
C784	R = 0.00597+0.00075*T	R=0.0356+(0.000874*T) - (0.00268*P)	82***
Bunya	R = -0.00427+0.00117*T	R=0.1130+(0.000122*T) - (0.00633*P)	89***

The regression equations (Table 3) were used to estimate the number of days from sowing to flowering for seven sites across eastern Australia for sowing dates of the first day of every month of the year using long-term average monthly temperatures. When flowering was predicted to occur between 35 and 60 days after sowing, the variety was considered to be potentially adapted to that location. A summary of sowing dates and cultivars with potential adaptation is summarised in Table 4.

Table 4. Potential planting date adaptation of four varieties of edamame soybean at seven locations across eastern Australia.							
	Atherton, Qld	Bowen, Qld	Bundaberg, Qld	Gatton, Qld	Richmond, NSW	Ballarat, Vic	Launceston, Tas
KS#1	?September	June to August	August	August to September	September to October	October to November	November
Tanbaguro	November to February	April to January	October to March	November to February	November to January	N/A	N/A
C784	September to March	April to March	September to March	October to February	? December	N/A	N/A
Bunya	September to March	April to March	September to March	October to February	October to February	? November	? November

The introduced varieties KS#1 and Tanbaguro had a relatively restricted range of planting dates to which they were potentially adapted.

KS#1 is a quick maturing cultivar. In the generally warm temperatures in most tropical and subtropical environments in Australia it flowers quickly and as a result produces inadequate vegetative growth to sustain reasonable pod yield potential. As a result hand harvesting costs would be high relative to the volume of product produced and mechanised harvest practically precluded by short height of pods above the ground.

Tanbaguro is a moderately daylength sensitive variety in terms of time to flower. This means that at earlier planting dates and in more temperate environments it is likely to be very late flowering, produce excessive vegetative growth and low pod yield. Conversely in the warm temperatures and shorter daylengths of the tropics, it will produce inadequate vegetative growth to sustain a reasonable pod yield. In subtropical environments, Tanbaguro is potentially adapted to late spring and summer planting.

Both Australian varieties C784 and Bunya show broad adaptation to planting dates and locations across eastern Australia. The cropping season for C784 and for Bunya would only be limited by cool temperatures in the winter in the subtropics. Further south, Bunya appears potentially adapted as far south as Richmond in the Sydney basin during the summer growing season.

Field-scale evaluation.

Evaluation of the variety C784 by cooperating growers was attempted at Kingsthorpe, Toogoolawah, Gatton, Eumundi and Walkamin in Qld and Lismore in NSW. Several of the sites experienced extreme difficulty in maintaining the crop due to shortage of irrigation water or attack from whitefly. However, produce of acceptable quality was produced at Eumundi and at Walkamin and was marketed via local farmers markets to ready acceptance by consumers. Trialling of C784 was halted due to the seriousness if its susceptibility to *Phytophthora* root rot. The variety Bunya has been trialled at Eumundi and was found to be acceptable to consumers.

Preliminary assessment of transport methodology and assurance of quality.

Following advice from Dr Sundar Shanmugasundarum of the Asian Vegetable Research and Development Corporation, a trial was conducted in which whole plants of C784 were picked early in the day, leaves removed, plants washed then the whole plant, with pods attached, packed in polystyrene boxes similar to the ones used for Broccoli. The boxes were packed with alternate layers of plants and shaved ice.

Representative boxes were retained in the CSIRO laboratory, dispatched to a vegetable freezing plant and to eight Japanese restaurants around Brisbane and Sydney.

Results and Discussion

After 48 hours in the laboratory, boxes were opened. Ice was still present and there was no apparent difference in appearance, flavour or texture compared with fresh- harvested product. Dry weight of seeds was 28 g/100 seed at harvest and was unchanged after storage.

Restaurant feedback was sparse, however all commented that the product was desirable, several commented that some of the seed size was small. This was perhaps not surprising given that 30 g/100 seed is a suggested minimum acceptable seed size.

The freezing plant commented that Edamame could be successfully frozen via a liquid nitrogen mediated technique but that they would need a larger sample to do a commercial scale evaluation.

2. Recommendations

1. Edamame should be gown as a vegetable crop under highly managed conditions, rather than as a grain crop in the way that soybean would normally be grown. Care should be taken to establish a plant population of around 5 to 10 plants per m^2 if using the cultivar Bunya, the crop should be maintained in very well watered condition and close attention paid to nutritional management and insect control to ensure that seed development is rapid and pods are unblemished. Seed size should be monitored though the latter stages of pod development, and harvest might reasonably commence once average fresh weight exceeds 60 grams per 100 seed.

2. The variety Bunya appears adequate to commence commercial development of the industry, however varieties with larger seed size and possibly also with improved sensory attributes would be desirable. Direct import of varieties from Asia or elsewhere, however, appears unlikely to produce a cultivar with acceptable adaptation to Australian conditions due to lack of climatic adaptation, lack of resistance to disease and extreme difficulty in seed production. Direct import of planting seed is precluded by Australian quarantine. It is however fortunate that the grain-soybean variety Bunya is also suitable for the production of edamame as this means that there is ample supplies of excellent quality planting seed.

3. At harvest time, care needs to be taken to ensure that seeds are large (over 30 grams/100 seed dry weight) and bright green in colour. If harvested at this stage of development, the vegetable appears robust enough to undergo freezing or transport under chilled conditions to distant markets. It also appears that there may be opportunities to develop supply relationships with specific high-end restaurants.

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