UNIVERSITI PUTRA MALAYSIA

GENETIC STUDIES IN EARLY GENERATIONS OF SOME AGRONOMIC AND QUALITY CHARACTERS OF VEGETABLE SOYBEAN (GLYCINE MAX (L.) MERRILL)

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DOCTOR OF PHILOSOPHY
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By

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Selection for large pods based on 100-seed weight were carried out in F_2 populations for four crosses, namely, Cross I (Kahori x AGS 186), Cross II (Kahori x AGS 187), Cross III (AGS 292 x AGS 186), and Cross IV (AGS 292 x AGS 187). The selected F_2 plants were then backcrossed to large seeded parents and the progenies, due to subsequent selfing, were achieved for further studies. Two experiments were conducted at Universiti Pertanian Malaysia (UPM), Malaysia and one experiment at Kasetsart University (KU), Thailand. The results of these studies showed that Kahori, AGS 292 and AGS 187 appeared to be potential parents for large pods. Furthermore, Kahori and AGS 187 were good sources for protein and oil while AGS 292 was a good source for sugar. AGS 186 was a good parent for high yield. The F_1 hybrids showed average mean values of pod size between the two parents. The progenies due to subsequent selfings from crosses between large pod and small pod varieties decreased pod size whereas crosses made among large pod varieties remained unchanged. These findings revealed that small seed varieties could

be improved by the backcross method using the large seed variety as the recurrent parent.

Based on the principal component analysis, there was evidence that Kahori, AGS 292 and AGS 187 were similar in terms of vegetative characters, yield. The F_1 hybrids from all crosses among them were close to the means between the respective parents involved and the F_2 populations were well segregated

Vegetable soybean grown at UPM, Malaysia gave higher yield than that grown at KU, Thailand due to the different growth conditions. There was an excessive rainfall during growing period of the trial conducted in Thailand. Nevertheless, good management of the crop at UPM probably was also the main reason for better pod yield.

The heritability values for yield components derived from the experiment conducted at UPM, Malaysia were lower than those derived from the experiment conducted at KU, Thailand. The high heritable characters were found in pod size both two- and three- seeded pod width, length and weight. Even though high heritability estimates were obtained for pod size, it would be ineffective to improve this trait due to the narrow genetic variability of the parents. Heritability estimates for quality characters were found relatively high for all crosses. In these studies, no significant amount of heterosis was found among the four crosses.

The improvement for large pods in early generations of selection would be achieved for the cross between Kahori and AGS 187. When hybridization was made with AGS 186, the high yielding variety (i.e. Kahori x AGS 186 and AGS 292 x AGS 186), the progenies had a tendency to produce small pods and slightly high yield. Hence, early generations of selection based on seed weight was unlikely to be practical for large pod size and high yield.

Abstrak disertasi yang di kemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat keperluan untuk ijazah Doktor Falsafah

KAJIAN GENETIK PADA GENERASI-GENERASI AWAL BEBERAPA CIRI AGRONOMI DAN KUALITI KACANG SOYA HIJAU (*Glycine max* (L.) Merrill)

Oleh

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Pemilihan untuk lengai besar berdasarkan berat 100-biji dilakukan pada populasi F_2 bagi empat kacukan, iaitu, Kacukan I (Kahori x AGS 186), Kacukan II (Kahori x AGS 187), Kacukan III (AGS 292 x AGS 186) dan Kacukan IV (AGS 292 x AGS 187). Pokok-pokok F_2 yang terpilih selanjutnya dikacukbalikkan pada induk masing-masing yang berbiji besar dan progeni-progeni terhasil daripada penyendirian seterusnya digunakan untuk kajian selanjutnya. Dua ujikaji dijalankan di Universiti Pertanian Malaysia (UPM), Malaysia dan satu ujikaji dijalankan di Universiti Kasetsart (KU), Thailand. Keputusan ujikaji-ujikaji tersebut menunjukkan bahawa Kahori, AGS 292 dan AGS 187 muncul sebagai induk yang berpotensi untuk menghasilkan lengai bersaiz besar. Juga, Kahori dan AGS 187 adalah sumber kandungan protin dan minyak yang baik, sementara AGS 292 adalah sumber yang baik untuk kandungan gula. AGS 186 adalah induk yang baik bagi hasil tinggi. Hibrid-hibrid F_1 menunjukkan nilai min purata bagi saiz lengai di

antara kedua-dua induk. Progeni-progeni daripada penyendirian seterusnya daripada kacukan di antara varieti-varieti lengai saiz besar dan lengai saiz kecil mengurangkan saiz lengai sementara kacukan di antara varieti-varieti saiz besar tidak berubah. Penemuan ini menunjukkan bahawa varieti berbiji kecil dapat ditingkatkan melalui kaedah kacukanbalik dengan menggunakan varieti berbiji besar sebagai induk.

Berdasarkan analisis komponen utama, dibuktikan bahawa Kahori, AGS 292 dan AGS 187 adalah bersamaan dari segi ciri-ciri tampang, saiz lengai dan hasil. Hibrid-hibrid F_1 daripada kesemua kacukan di antara mereka adalah hampir dengan min di antara induk masing-masing dan populasi F_2 bersegregasi dengan baik.

Kacang soya hijau yang ditanam di UPM, Malaysia memberi hasil yang lebih tinggi daripada yang ditanam di KU, Thailand disebabkan oleh perbezaan keadaan penanaman dan terdapat hujan yang berlebihan sepanjang tempoh penanaman bagi ujikaji di Thailand. Pengurusan tanaman yang lebih baik di UPM mungkin juga menjali penyebab utama bagi hasil yang tinggi.

Nilai kebolehwarisan bagi komponen-komponen hasil daripada ujikaji di UPM, Malaysia adalah lebih rendah daripada yang diperoleh daripada ujikaji di KU, Thailand. Ciri-ciri yang mempunyai kebolehwarisan tinggi didapati pada saiz lengai, berbiji-dua dan juga berbiji-tiga, lebar, panjang dan berat lengai. Walaupun jangkaan kebolehwarisan diperoleh dari saiz lengai adalah tinggi, untuk meningkatkan ciri ini tidak akan berkesan disebabkan kepelbagaian genetik yang sempit di kalangan induk. Jangkaan kebolehwarisan bagi ciri-ciri kualiti adalah agak tinggi bagi kesemua kacukan. Dalam kajian-kajian tersebut, tidak terdapat nilai heterosis yang bermakna di antara ke empat-empat kacukan.

Lengai bersaiz besar dapat dihasilkan pada pemilihan generasi awal dari kecukan di antara Kahori dan AGS 187. Apabila penghibridan dilakukan dengan AGS 186, varieti berhasil tinggi (Kahori x AGS 186 dan AGS 292 x AGS 186) progeni-progeni tersebut cenderong mengeluarkan lengai kecil dan hasil sedikit tinggi. Justeru itu, pemilihan generasi awal berdasarkan berat biji tidak mungkin berkesan untuk mendapatkan lengai besar dan hasil tinggi.

CHAPTER I

INTRODUCTION

The soybean (*Glycine max* (L.) Merrill) can be categorized into grain soybean and vegetable soybean depending on its form of utilization and nutritive value (Martin, 1984). The grain soybean normally has small seed size and it is normally consumed as processed products such as soybean milk, soybean oil, bean curd and fermented soybean. It is a good source for protein and oil. The protein meal can be processed and used as meat substitutes for human diet and as a supplement for animal feeds. The oil extracted from soybean can be used for human consumption and for industrial purposes. For human consumption, it is mainly used as cooking oil, salad dressing and margarine. For industrial purposes, soybean oil can be used to manufacture paints, oil clothes, printing inks, soaps, insecticides and disinfectants (Purseglove, 1968; Chapman and Carter, 1976).

When the soybean is consumed as a vegetable or snack food, the seed is preferable to be large. The vegetable soybean seed is commonly used as a source of protein or a diet food in many Southeast Asian countries (Japan,
China). In Japan, immature green pods are boiled with salt and served as a snack food in pubs. In Taiwan and Korea,
various kinds of meat. In addition, it can also be served as a useful medicine for some diseases such as gastric fever, bladder trouble and improper circulation of blood (Shanmugasundaram et al., 1989; Lumpkin and Konovsky, 1991).

Considering its nutritive value, vegetable soybean is one of the most nutritious crops providing essential vitamins, minerals and protein. There is a great potential to use vegetable soybean as a substitute for meat protein in developing countries (Shanmugasundaram et al., 1989). However, as a snack food, it is quite popular because of its high sucrose, glutamic acid and alanine contents that make it a very palatable food (Lumpkin and Konovsky, 1991).

The vegetable soybean has been consumed by human beings for a long time in Japan, Taiwan,

Among these countries, Japan produces and consumes the largest amount of vegetable soybean (Martin, 1984; Shanmugasundaram et al., 1989). The soybean produced in Japan is usually of high quality with good appearance. The criteria for the Japanese market are large pods with two or three seeds per pod, bright green colour, spotless surface and good taste (Chiba, 1991;

being, the production in Japan cannot cope with the demand. Therefore, fresh and frozen vegetable soybeans have to be imported from Taiwan (accounting for 99%), Mainland China, Thailand and Mexico (Nakano, 1991). The production area in Taiwan, however, has been reduced recently because of high production cost and shortage of labour for the cultivation. Hence, Thailand,

Malaysia have emerged and they may become the new vegetable soybean producing countries aiming for the Japanese market (Cheng, 1991; Shanmugasundaram et al., 1991).

At present, vegetable soybean is the new commodity in Malaysia and we are looking for adaptable varieties for the farmer to grow the crop locally. In 1987, the Malaysian Agricultural Research and Development Institute (MARDI) evaluated some commercial varieties from the Asian Vegetable Research and Development Center (AVRDC), Taiwan and Japan under Malaysian conditions. Based on the results obtained, it was noticed that AGS 190 was the most suitable variety for local conditions in terms of growth and yield (Ramli and Ibrahim, 1991). In 1992, 15 lines from AVRDC and three lines from Thailand have been evaluated at the research farm at the Universiti Pertanian Malaysia, Serdang, Selangor. The results showed that AGS 292, Kahori and Shironomai produced acceptable seed size but they were low yielders whereas AGS 186 gave high yield but the seed was small (Tang, 1992; Chew, 1993). Hence, a breeding programme through crossing has been established aiming to develop suitable varieties for the Malaysian environment.

In this research, the inheritance and degree of heterosis of agronomic characters, protein, oil and total soluble solids are examined. These studies, especially on seed quality are very important for developing high quality vegetable soybean genotypes. Furthermore, in planning a breeding programme, it is also important to evaluate the improvement of correlated characters in early generations of selection in the process of developing superior genotypes. Hence, the objectives of these studies were:

(1) to evaluate the agronomic performance of parental lines, the F_1 hybrids and the selected and unselected F_3 and F_4 populations.
(2) to examine the heritability and degree of heterosis of agronomic and quality characters.
(3) to determine the improvement of correlated characters in early generations of selection.

CHAPTER II

LITERATURE REVIEW

Botany

The vegetable soybean is a type of grain soybean (*Glycine max* (L.) Merrill) harvested and consumed as a form of vegetable. It is originated in northern China. It is an important food crop in China, Korea, Japan and other countries in Southeast Asia.

The vegetable soybean is an annual short-day plant. The plant is erect, bushy and leafy. Most of the vegetable soybean varieties are determinates where the stem growth will cease at flowering stage. The mature plant has 7-13 nodes which were completely differentiated in 4-5 weeks after planting. Two to three branches are developed only at the lower nodes of the plant. Plant height is about 30-70 cm with simple leaves arranged in opposite side at the first node. The other leaves are trifoliate and alternate in arrangement. There is tawny or gray coloured pubescence on the stem, leaf and pod.

The flowers of vegetable soybean are perfect flowers and self-fertile. They are born in axillary and terminal racemes. The colour of flower is white or purple depending on variety. The corolla consists of five petals, namely; one standard, two wings and two keels. The androecium comprises ten stamens. The filament consists of nine stamens developed in a tube around the pistil. The ovary is monocarpellate.

The immature pods are harvested at the stage when the seed is fully developed. The pod colour can be green with white, brown or gray pubescence. The seed coat colour may be yellow, green, brown or black. The number of seeds per pod ranges from one to four. Seed size is large in which one-hundred seed weight is 30 g or more (Fehr and Caviness, 1977: Poehlman, 1979; Shanmugasundaram et al., 1991).

Status of Vegetable Soybean Cultivation

The main production areas of vegetable soybean are Japan and Taiwan. Japan produced 104,500 t of vegetable soybean from 14,400 ha in 1988 but the domestic consumption was about 110,000 t (Masuda, 1991; Nakano, 1991). The balance of the demand was imported from Taiwan valued at about US$ 48.1 million (Chiba, 1991). Taiwan had about 9,852 ha of vegetable soybean with the production of 63,163 t in 1990. A majority of the production were exported to Japan, accounting for 80% of the total. However, in recent years the production has been moved to Thailand, Philippines, Indonesia and Malaysia due to a high cost of production of vegetable soybean in Taiwan. Since vegetable soybean produced in Taiwan has been recognised as a good quality product, vegetable soybean produced in other countries aiming for the Japanese market should also reach the required standard (Shanmugasundaram et al., 1991).

In Japan, soybean was introduced from Korea and Shanghai during the Yayoi period (200 B.C. to 250 A.D.). The immature beans have been used as the sacred food. However, it is normally consumed as a snack with beer which is very popular among the Japanese people.

The demand of vegetable soybean is high in summer seasons. Tohoku and Kanto districts are the main production areas during September to October. In winter and spring seasons, the planting area is Shizuoka prefecture which produces the largest quantity of vegetable soybean in the greenhouse cultivation system. The domestic production is distributed as fresh vegetable soybean. However, during

December to June when the production in Japan is very little and the price is high, frozen and fresh vegetable soybeans are imported from Taiwan (Nakano, 1991).

The Asian Vegetable Research and Development Center (AVRDC) in Taiwan introduced soybean germplasm from Japan in 1957. Germplasm collection has been screened and selected for large seeds and high pod yield since 1965. Based on the regional yield trials carried by the Kaohsiung District Agricultural Improvement Station, the pure line selection from Taisho Shiroge or AGS 292 was selected and released as Kaohsiung No. 1 (KS # 1) in 1987. This variety became very popular among growers and consumers, and therefore, the planting area was increased from 46% of the total acreage in 1988 to 84% in 1990 with the exported value amounted to US$ 63 million (Shanmugasundaram et al., 1991). About 60% of the production was exported through Japanese trading firms, 20% purchased by Japanese manufacturers and the rest 20% was sold directly to Japanese supermarkets and other buyers. The fresh vegetable soybean, sometimes, was airlifted to Japan in the morning and sold in Tokyo supermarkets in the evening of the same day (Liu and Shanmugasundaram, 1984).

In Taiwan, the vegetable soybean is grown in the rice bunds either using the non-tillage or tillage methods. The non-tillage method is used only at Pingtung and Kaohsiung areas during the autumn season after the second rice crop. The tillage method is used in wider areas during various seasons of the year (Liu and Shanmugasundaram, 1984).

In Thailand, vegetable soybean is not the new commodity crop. It has been consumed for many years and grown as supplement crop to rice, corn and cassava. The planted areas, in the past, were not isolated because the production either for grain or for fresh pods were from the same variety. Recently, the private sector introduced vegetable soybean seeds from Japan and Taiwan and distributed to the farmers to grow for the export market in Japan. In consequence, growing soybean for fresh pods has become specialized and has restricted to certain areas only (Chainuvati, 1992).

The production areas are mainly in the central and northern regions of Thailand. In the central region of Nakhon Pathom and Ratchaburi provinces, vegetable soybeans can be grown in all seasons but the production is higher during the cool season compared to other seasons. The most suitable period for growing both vegetable and grain soybeans is between October and December. In the northern region of Chiang Mai province, vegetable soybean may be grown for two seasons continuously. The first crop is grown in September and the second in December by using the seed from the first crop (Sitadhani, 1992).

Research on improvement of vegetable soybean varieties is carried out by Chiang Mai Field Crop Research Center (CMFCR), Chiang Mai and the Tropical Vegetable Research Center (TVRC), Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom province.

Vegetable soybean varieties were introduced from Japan and Taiwan in 1986 by CMFCR. Based on location trials, VESOY # 4 or also known as TVB 1 (Thai Vegetable Soybean No. 1) was suitable for local consumption (Chotiyarnwong, 1992).

At the Tropical Vegetable Research Center, most vegetable soybean varieties are also imported from Japan and Taiwan. More than 20 strains have been tested and evaluated. Only seven varieties were found to be of high potential to grow in Thailand. There were AGS 292 or Kaohsiung No. 1, Tsuzunoko, Wakakusa, Kegon, Karitea, Fukuichi and White Lion. All these varieties had high yield with green pods and white pubescence. However, the most promising variety for the central region is AGS 292 (Sitadhani, 1992).

In Malaysia, the production of soybean is limited although the domestic demand is high. There are no adapted varieties that can be grown under humid tropical climate like Malaysia. Moreover, the poor seed quality, weed, pest and disease are the main problems in soybean cultivation. Soybean and its products, therefore, are imported (Yap, 1986). However, soybeans have been introduced from United States, SEARCA (Southeast Asian Regional Center for Graduate Study and Research in Agriculture) and Taiwan to Malaysia to grow as an intercrop in immature rubber and oil palm plantations or as a sole crop in rotation with rice and other annual crops (Abu Kassim, 1976; Yap, 1986). Due to high production cost and requirement for more intensive care of soybean cultivation, there is still no large scale planting of soybean in Malaysia (Mak, 1986).

Quality of Vegetable Soybean

The quality of vegetable soybean can be classified into marketing criteria and chemical compositions which comprise eating quality and nutritive values.

Marketing Criteria

Marketing criteria or appearance characteristics of vegetable soybean are the major quality requirements by consumers. The characters consist of pod size, pod colour, pubescence colour, number of seeds per pod and seed size. These characters cannot be changed by the producers, therefore, grading the pods to meet the required specifications can be done after harvesting. Ungraded pods which will be removed are yellow pods, pods with insect damage, sunburn pods and pods with other defects. The standard criteria for vegetable soybean exported to Japan are large pods and seeds. Pod width and pod length should be more than 1.4 cm and 5.0 cm, respectively. Two or more seeds per pod are preferred. Pods should be bright green colour after blanching or cooking with gray pubescence. In 500 g, the pods should not be more than 175 pods (Shanmugasundaram et al., 1991).

Chemical Composition

Chemical composition comprises eating quality and nutritive values. These two qualities are the desired characters that can be changed according to the preference of the consumers.

Eating Quality

The eating quality of vegetable soybean consists of sweetness, taste, texture and flavour. Sugar and amino acid contents in seeds are responsible for the sweetness and taste while the lipoxygenase enzyme system is responsible for the beany flavour (Takahashi, 1991).

Sweetness : Starch and sugar contents in seeds are responsible for soybean sweetness. Starch content changes with accord to maturity of the pod and it reaches the maximum content at the mid-pod-filling stage and it decreases after this stage. The starch accumulation may be changed to sucrose by starch synthesis enzymes (ADP-glucose pyrophosphorylase, branching Q enzyme and sucrose synthase). The sucrose level increases at the green pod stage or early development stage about 35 days after flowering. Even though the vegetable soybean has more sucrose content than the grain soybean but the level of the total sugar does not exceed 6% on the fresh weight basis (Masuda, 1991). According to Tsou and Hong (1991), starch and sucrose contents in vegetable soybean are higher but oligosaccharide content is lower than that in grain soybean (Table 1). Low oligosaccharide content is the preference of consumers because it is responsible for the flatulence experienced by human taste (Openshaw and Hadley, 1978).