Improving Seed Viability in Vegetable Soybean

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THE WORLD FOOD PRIZE

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Title: Improving Seed Viability in Vegetable Soybean

Abstract:

Loss of seed viability is one of the major challenges faced by vegetable soybean growers. An experiment conducted with vegetable soybean lines from AVRDC South Asia showed good variation for germination ability, seed size and seed weight. A grain soybean line (GC-84501-32-1) with high germination ability was identified for further testing and for future use as a donor parent in vegetable soybean improvement program. The study on a segregating population (F_2) from the cross between GC-84501-32-1 and AGS 406, a vegetable soybean with larger seed size confirmed the true nature of the cross. Selection for high germination ability may be carried out in the F_3 generation on a plant to row progeny basis.

Introduction:

Goals, aspirations, hopes, and dreams have always been a driving force. They are what enable humans to push forward even when faced with the seemingly impossible. In 2014, while attending the Global Youth Institute, a challenge was issued out. How can we feed a world with an estimated nine billion people by the year 2050? This challenge became and is still becoming the goal and dream for many, including myself.

A land rich in culture and history, India is still a developing nation due to many issues such as vast geography, low agriculture productivity, and a rapid growing population. The land ranges from the wintery mountains in the North to the hot, dry plains in the South. About half of the population has an agriculture related occupation, yet, it is estimated that about a third of Indians are malnourished (Malnutrition Health Issues India, 2012). Ranking number two in population in the world, India has approximately 1.27 billion people (India Population 'LIVE', 2014). India has come a long way from the impoverished place it used to be, but still has even farther to go to completely eradicate hunger and poverty. Millennium Development Goals says India has been the biggest contributor to poverty reduction between 2008 and 2011, with around 140 million or so lifted out of absolute poverty (Boopathy, 2014). Even so, 29.8% of India's population is still below the poverty line (Southeast Asia: India, 2010).

India has considerable amount of unlocked potential in agriculture. India was ranked as one of the top 3 producers in the world (FAOSTAT, 2013; Fig 1). Yet, India has 31% of the world's children who are stunted and 42% of those who are underweight (Sankar and van den Briel, 2014). With many children and adults affected by micronutrient deficiencies, India has great need to improve the quality and nutrition of their diets (GAIN, 2014). One approach is to introduce, or improve nutritional crops to the greater Indian population. A promising crop, Vegetable soybean, is rich in protein and other nutrients, and could potentially be a healthy and nutritional addition to the Indian diet.

India is the top country in the world that holds the most vegetarians. It is estimated 42 percent of households are vegetarian based on the National Sample Survey (NSS) (Delgado, 2003). Vegetarian is defined by that they never eat fish, meat or eggs. The other 58 percent are a mixture of less strict vegetarians or non-vegetarians (Delgado, 2003). This is about half a billion people living on mostly grains and vegetables alone making it the largest group in one area. The choice of cutting out fish, meat, and eggs out of their diet is mainly driven by class and religious systems in India ("500 Million Vegetarians in India", 2013).

Along with being the top vegetarian nation in the world, India also has the highest rate of anemia causing several health issues. Fifty percent of India's population is affected by anemia. This is more prevalent in women than in men. According to WHO (2000), if the affected rate of anemia is greater than 40 percent, then it is considered of high magnitude. Twenty to forty percent of maternal deaths are due to anemia. The most common nutrition factor causing anemia is iron deficiency (Kaur, 2014).

The major source of proteins for the vegetarian population in India is legumes. Mahatma Gandhi introduced the use of soybeans as a low cost, high quality protein food in India in 1935. Due to long cooking and disliking the distinct beany flavor, grain soybean remained unpopular therefore mostly unknown in the Indian population. Vegetable soybean (*Edamame*) was introduced as substitute instead of the unpopular grain soybean. Vegetable soybean is characterized by dark green pod and bean color. The higher sucrose levels in vegetable soybeans cause them to have a sweet taste instead of a beany flavor. Edamame is rich in protein, unsaturated fat, phosphorous, iron, thiamine, riboflavin, and calcium compared to the other legumes in the Indian diets, and can help enrich the nutrition of Indian people's diets (Shanmugasundaram *et al*., 2015).

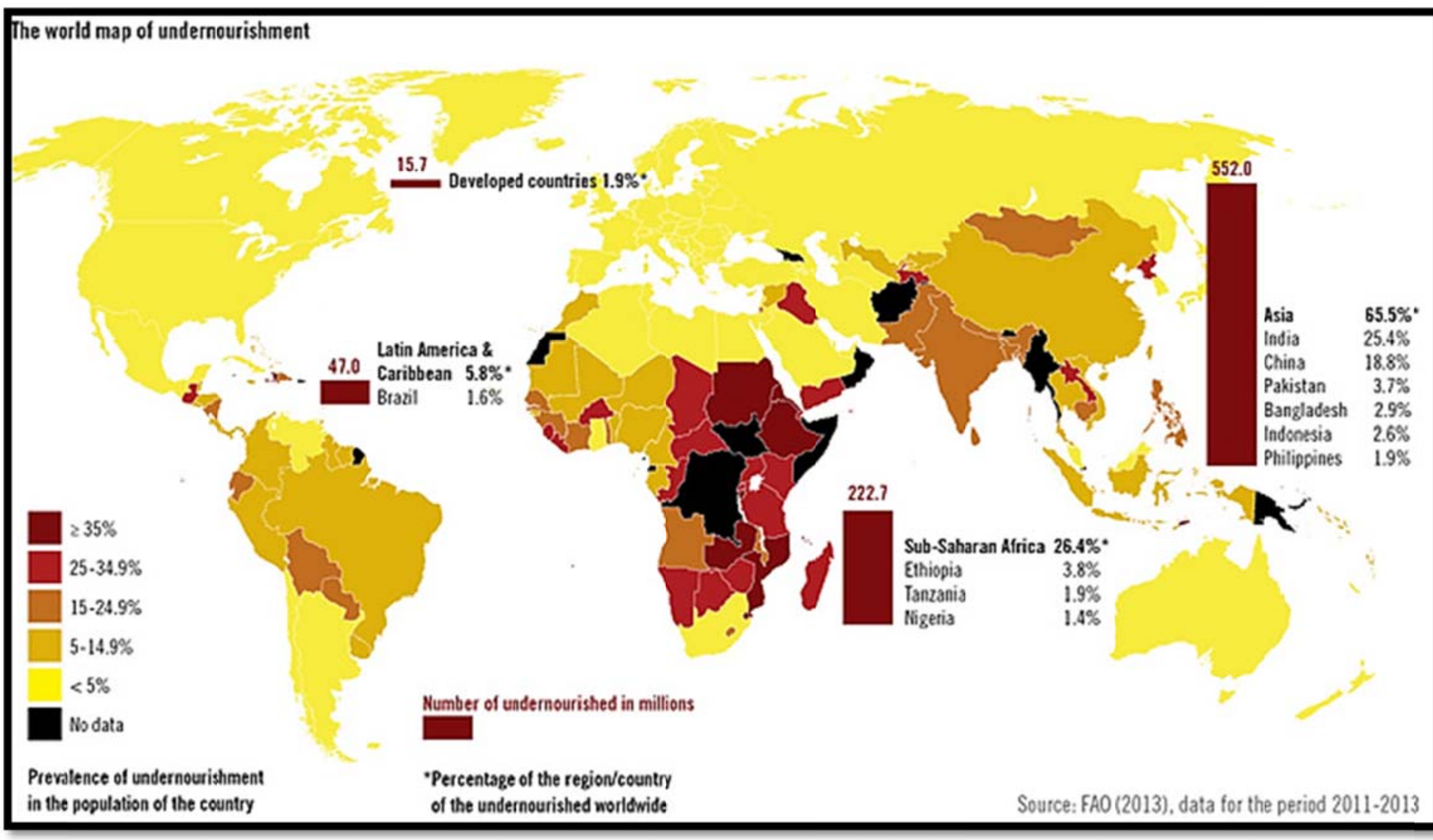


Fig 1. Derived from the 2013 FAO World Fact Book shows India is portrayed with having a relatively high undernourishment rate.

Background:

AVRDC-The World Vegetable Center

“Prosperity for the poor, and health for all”

The AVRDC began on 22 of May, 1971 in Taiwan. Originally it was the Asian Vegetable Research and Development Center, but changed its name to the World Vegetable Center in 2008 as it grew globally. From the beginning, the AVRDC has been tasked to fight against malnutrition and poverty by developing, improving, and increasing production and nutrition of vegetables as well as promoting them. As a multi government funded organization they work closely with the private sector in postharvest processing and integrated pest management. Along with prestigious research and creating public relations, AVRDC is home to the world’s largest public collection of vegetable germplasm. They have more than 61,435 accessions of 439 species from 155 countries stored. AVRDC distributes approximately 10,000 seed samples to researchers across the planet every year. They have released hundreds of new vegetable varieties with a focus in developing countries. Examples of some of their achievements include developing varieties of tomatoes with 3 to 6 times the normal amount of Vitamin A, disease-resistant mungbeans, high yield, and heat-tolerant sweet peppers. They also develop sustainable “home garden” system models, which are vegetable gardens designed to sustain one family for a year with complete nutritional needs.

My supervisor Dr. Ramakrishnan Nair, Vegetable Breeder at AVRDC, and I in front of AVRDC South Asia.

Literature Review:

Introduction:

Vegetable soybeans (*Edamame*) are harvested at the R_6 stage when the pods are still green. Vegetable soybeans are classified to have a large seed size (> 30g/100 seeds on dry weight basis) compared to grain soybeans. They are traditionally consumed in China, Japan, Korea, Taiwan, and Thailand. Vegetable soybean is usually characterized by dark green pod and bean color, but seed coat color can also include yellow, brown, or black. Vegetable soybean has no undesirable beany flavor unlike grain soybean, and with many nutritional benefits it is becoming a trendy health food. Vegetable soybean is being marketed as fresh pods detached from the stem, fresh shelled green beans, frozen pods, frozen shelled green beans, and dry roasted green beans (Shanmugasundaram *et al*., 2015). It is usually prepared by boiling and lightly salting the pods and then serving as a snack accompanied with an alcoholic beverage such as beer.

Table 1. Vegetable soybean compared to similar cooking legumes

Legume (100g)	Energy (kcal)	Protein (g)	Total Lipid (g)	Dietary Fiber (g)	Sugar (g)	Calcium (mg)	Iron (mg)	Zinc (g)
Edamame, frozen, unprepared	110	10.25	4.73	4.8	2.48	60	2.11	1.32
Peas, green, frozen, unprepared	77	5.22	0.40	4.5	5.00	22	1.53	0.82
Pigeon peas, immature seeds, raw	136	7.2	1.64	5.1	3.00	42	1.60	1.04
Lima beans, immature seeds, frozen, unprepared	106	6.40	0.35	5.5	1.39	24	1.51	0.49

Values obtained from the USDA National Nutrient Database for Standard References, Release 27 (2011).

The field performance of soybeans is heavily dependent on seed quality. Two attributes of seed quality that are often measured are seed viability and vigor (Johnson and Wax, 1978). The viability of a seed refers to its ability to germinate and produce a normal seedling (Delouche and Caldwell, 1960). According to the Association of Official Seed Analysts (AOSA, 2009) seed germination is 'the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions'. Smith (2010) reported that ideal genotypes for seed quality would have standard and accelerated aging germination of greater than or equal to 90%.

Seeds that have high initial viability withstand unfavorable storage conditions better than similar seeds of low initial viability. The predictive ability of any seed quality test of seed deterioration in storage is based on the relationship that exists between the initial seed quality, seed longevity, seed moisture content and storage conditions of temperature, relative humidity and oxygen concentration (Tang *et al*., 2000). Several inherent (genetic) factors of the seed such as hybrid vigor, hard-seededness, susceptibility to seed damage, and chemical composition can influence the seed vigor and ultimately, viability (Copeland and McDonald, 2001).

Factors Affecting Seed Viability:

Storage Conditions

Seed storage is influenced by the initial quality of the seed lot, the moisture content of the seed, and the temperature, relative humidity and gaseous exchanges in the storage environment (Barton, 1943; Vertucci and Roos, 1990; Vertucci and Roos, 1993). Maximum seed quality, as defined by seed germination and vigor, coincides with the developmental stage of physiological maturity (Bewley and Black, 1994). Beyond this stage the seed starts deteriorating. Seed deterioration is an inexorable process that cannot be reversed. Only its rate can be slowed by storage in a controlled environment (Delouche, 1968).

Moisture

The relative importance of each factor to the rate of deterioration of a seed will depend very much on the moisture content of the seed (Harrington, 1973).The rate of deteriorative reactions in a seed increases with an increase in its moisture content (Delouche, 1968). Seeds with moisture contents in the range of 4-6% to 12-14% store longer than seeds with moisture contents above and below this range (Harrington, 1973).

Temperature

Temperature can control the rate of which reactions occur in soybean seeds. High temperatures are known to increase the rate of reactions by affecting the enzymes that are involved in reactive oxygen species scavenging and repair (Bernal-Lugo and Leopold, 1998). One study concluded that seeds stored at 5°C had higher germination than seeds stored at 25°C, but 25°C were still significantly superior than seed stored at room temperature (Singh and Gunasena, 1974).

Another study showed that seeds with higher water content (18%) stored at lower temperature such as 5°C could extend the aging effect (Hou, 2004.) Soybean seeds stored for 6 months at a temperature of 15°C maintained high germination and vigor, and the germination rate remained at 95% for 6 months when a cool storage environment also was maintained at 60% relative humidity (Krittigamas *et al*., 2001). Other studies showed that seeds stored in controlled temperature of 15-20 °C had higher percentage germination than those stored at ambient temperature (Nattasik *et al*., 2001).

Humidity

The decline in viability is intricately linked to the moisture content of the seed, which is in turn controlled by the relative humidity of the storage environment (Barton, 1943; Vieira *et al*., 2001). The rates of thermal-chemical reactions within the seed will increase when the relative humidity of its environment was 27%, but at a relative humidity of 19%, physiological reactions were slowed down enough for optimum longevity of the seed (Vertucci and Roos, 1990) Therefore, the optimum moisture content for seed storage in terms of relative humidity was defined as the moisture level between the relative humidity where reactions become thermodynamically less feasible due to slow diffusion, and the level below which seeds deteriorate more rapidly. The moisture content of the seed that is in equilibrium with relative humidity of between 19 and 27% was defined as the optimum for storage (Vertucci and Roos, 1990).

Pest and Disease

Pests and diseases are some of the major issues affecting seed viability. Pathogens found on or in seeds that could potentially be transmitted to a subsequent crop can adversely affect germination and vigor (Kulik and Schoen, 1981; Hepperly and Sinclair, 1982), emergence and seedling vigor. The major disease to effect vegetable soybean is rust (*Phakopsora pachyrhizi* Syd), which detrimentally effects seed/pod quality and yield. Typical pests for vegetable soybean include beanfly, whitefly and the pod borer. Soybeans are highly susceptible to fungal infections which cause loss in seed viability. Fungal invasion of seeds can lead to destruction of cell walls, starch granules, and storage proteins (Nightingale *et al*., 1999; Lisker *et al*., 1985). These effects have been documented in most seeds of cereal crops infected with Fusarium spp. (Nightingale *et al*., 1999). Fungi such as *Colletotrichum truncatum* (Schw.) Andrus & W. D. Moore, *Phomopsis sojae* Lehman, *Cercospora kikuchii* (Tak. Matsumoto & Tomoy.) M. W. Gardner and *Alternaria* spp. are considered seedborne and usually infect the seed in the field (Schortt *et al*., 1981; McGee, 1995). Seed infected with Charcoal rot often carries the fungus on its seed coat and reduces germination (Integrated Crop Management, University of Iowa, 2010). Warm and wet conditions are known to increase infection by some fungi especially *Phomopsis longicolla* on soybean (Spears *et al*., 1997). Lisker *et al*., (1985) found that seeds with cracked seed coats had more fungi than seeds with intact seed coats. Fungi commonly referred to as storage molds belong to the genera of Chaetomium sp., Rhizopus, Mucor, *Aspergillus* and *Penicillium* spp and increase during storage (Roy *et al*., 2000). In one study, infection rate of soybean seeds with *Diaporthe phaseolorum* (Cooke Ellis) Sacc. var. *sojae* was significantly lowered after 2 years of storage in a dry and cool environment (Kulik and Schoen, 1981). Diseased plants in the field produce shrunken seeds and often times smaller seeds that have reduced germination potentials and low vigor (McGee, 1995).

Duration

It is a common problem that soybean seeds do not store long term. Delouche (1968) defined the total seed storage period as comprising segments of bulk storage, which is the period from harvest through packaging including conditioning. One study found that there was no significant difference between seed germination in different varieties for the first 6 months (Singh and Gunasena, 1974). Soybeans are not carried over for planting beyond six months because of the fast rate at which the seeds age and eventually lose their ability to germinate (Burris, 1980; Byrd and Delouche, 1971). After 6 months, one study found that seeds stored at 5°C maintained higher germination (Singh and Gunasena, 1974). It was hypothesized that treated soybeans seeds could be carried-over at least two years if the storage conditions follow Harrington's rule (1959) of temperature of 10°C) and 50% relative humidity (Mbofung, 2012). Another study, Burris (1980), suggested that drying soybean seeds down to 8-10% moisture level before storing at low temperatures and relative humidity could provide acceptable seed quality for at least 3 years.

Chemical Composition:

Chemical composition has a substantial variation. The chemical composition of the seed determines the optimum seed content factors, which varies among varieties, among species, among cultivars and among tissue types in the individual seed (Vertucci and Roos, 1990).

Starch

Starch content changes with maturity, peaking at around the mid-pod-filling stage at 22%. High-starch varieties such as Koito-zairai and Tururan-daizu have been identified (Masuda, 1991). α-amylase activity is known to be present in mature soybean seeds which are positively associated with the germination of soybean seeds (Thomas, 1979).

Sugars

Soybean seeds with high sucrose are found to be more desired because it improves taste (Hou *et al.*, 2009). The late embryogenesis abundant proteins and oligosaccharides form the glass that maintains the macromolecular structure of the seed (Tang *et al.*, 2000; Wang *et al.*, 2000; Sun *et* *al*., 2007).The amount of high temperature oligosaccharides within a seed influences the stability and magnitude of the glass state. Therefore, the higher the oligosaccharide content, the greater the stability of the glass and the longer the soybean seed could be stored (Bernal-Lugo and Leopold, 1995)

Protein

Soybeans are high protein crops. Protein in soybean seed ranges from 34 to 57% of the total seed weight (Bellaloui *et al*., 2011). Together, these soybean storage proteins account for about 70% of the total protein content in the seed (Morales and Kokini, 1997). Other studies have found that high protein levels in soybean seeds were correlated with lower seed germination percentages irrespective of the moisture contents of the seed in the laboratory (LeVan *et al*., 2008)

Lipids

Twenty percent of the dry weight of soybean seed is made up of oil (Clemente and Cahoon, 2009). The oil content ranges from 8.3 to 28%, with a mean of 19.5% (Wilson and Rinne, 2004). The soybean seed contains 14 fatty acids of which palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linoleic (C18:3) are considered essential to human nutrition. The concentration of saturated fatty acids in soybean oil ranges from 10 to 12% palmitic acid (C16:0) and 2.2 to 7.2% stearic acid (C18:3) (Cherry et al., 1985). The mean concentration of unsaturated fatty acids in oil is 24% oleic acid (C18:1), 54% linoleic acid (C18:2), and 8.0% linoleic acid (C18:3) (Schnebly *et al.,* 1994). One study found that deteriorative changes during storage might be related to higher lipid content (Sharma *et al*, 2011). While another study showed that higher oleic acid and lower linoleic acid are desirable traits for oil stability and long-term shelf storage (Clemente and Cahoon, 2009).

Strategies to Improve Viability:

The use of plant genetic resources in crop improvement followed by adoption, cultivation and consumption of the improved cultivars by farmers, is one of the most sustainable methods to conserve valuable genetic resources for the future, and simultaneously to increase agricultural production and food security (Haussmann *et al*., 2003). Seed longevity in storage is also a genetically regulated process because the accumulation of seed storage substances is the result of a determinant genetic program (Delouche, 1968).

High Germinating Lines

Seed vigor differences exist among species and among cultivars of the same species; and these seed vigor differences are genetically regulated (Yu et al., 1999). With a wide range of genetic variation, the need to select high germinating elite soybean lines arises. With high germinating lines in mind, selective breeding can be done for desired traits and improved quality and nutrition.

Breeding

Genetic resources can be defined as all materials that are available for improvement of a cultivated plant species (Becker, 1993). Traditional breeding has been underway to develop cultivars with higher nutrition. The optimal percentage of the exotic plant genetic resources genome (100%, 50% or 25%) in a breeder's population depends on the overall objective, available time-frame and finances, the level of adaptation of the plant genetic resources, and the yield difference between plant genetic resources and actual breeding population (Haussmann *et al*, 2003). Hartwig *et al*. (1997) suggested that there is a possibility for developing soybean germplasm with high seed protein and lower stachyose + raffinose in the meal. In their experiment, they evaluated sucrose, raffinose, and stachyose in 20 soybean cultivars and breeding lines with high oil and 20 breeding lines with high protein and found non-significant negative correlation between protein and stachyose + raffinose, suggesting sugars in soybeans seed vary among soybean genotypes and between maturity groups (Hymowitz *et al*., 1972; Hymowitz and Collins, 1974; Hartwig *et al*., 1997; Hou *et al*., 2009). They found significant variability among genotype (Hymowitz, 1972). Other researchers evaluated 195 soybean cultivars, and concluded that sufficient variability exists for sugar selection (Hymowitz and Collins, 1974). If selective breeding can be done for sugar content, then it could also be possible to selective breed for high germination as many of those traits (oils, sugar, and protein) take effect on seed viability.

Conclusion:

Vegetable soybeans are becoming an upcoming and increasingly popular nutritious crop in other parts of the world despite being used in south East Asia for years. They are high in protein and many other nutrients that would make it a successful crop in the fight against malnutrition. Many factors such as storage conditions and seed composition take effect on soybean seed viability. These factors must be taken into consideration when selecting breeding populations. Great potential can be unlocked through successful breeding programs. With good management practices, and successful breeding programs implemented these issues effecting seed viability can be overcome to develop a crop that is both high quality and nutritious.

Experiment Report:

Introduction

Vegetable soybean is a type of soybean (*Glycine max* (L.) Merr.) in which the immature pod is harvested and used as a fresh or frozen vegetable. Immature pods are boiled and the seeds extracted as a highly nutritious snack food. It is popularly known as Edamame in Japan, *Maodou* in China and green soybean in North America. Vegetable soybean seeds are larger (over 30g/100 seeds), have a milder flavor, nuttier texture and are easier to cook in comparison to grain soybean. It is widely grown and used in East Asia, where it is believed to have been domesticated. Because of its high nutritive value, the crop is becoming popular in other regions of the world (Shanmugasundaram *et al*., 2015).

One of the challenges faced by vegetable soybean farmers is the quick loss of seed viability. Hence this is an area of great interest to breeders and growers for vegetable soybean improvement.

Objectives

In this study our objectives were:

1) To test the germination ability of elite vegetable soybean lines developed at AVRDC.
2) To verify the association between seed size and germination.
3) Explore the possibility for improved germination by breeding.

Materials and Methods

Experiment No. 1

Vegetable soybean *Glycine max* (L.) Merr.) lines (13) of varying seed colors plus one grain soybean available at AVRDC- The World Vegetable Center, Hyderabad, India were utilized for the study (Fig. 1). The source of the seed was from a previous crop, stored for about 60 days after harvesting. Twenty five seeds of each of the 14 lines were selected for the experiment. Five replicates were conducted for each of the 14 lines. Prior to the start of the experiment, the seed size of all the lines were measured with the help of a Vernier Caliper (Fig. 2). In addition weight of five seeds was measured using a balance. A completely randomized design was used for this experiment (Fig. 3). In each petri dish, Whatman 42 filter paper was placed and five seeds of each line was sown. About 2ml of distilled water was then added. Duration of this experiment lasted for five days at temperature of 25°C. Each day the seeds were observed for germination, and the data were recorded. An analysis of variance for three traits namely percentage of germination (transformed using square root transformation), seed size and seed weight was conducted using GenStat® software. In addition the association between the seed traits and percentage of germination was determined.

Experiment No. 2

In a previous study conducted at AVRDC South Asia, a soybean line with good germination ability (GC-84501-32-1) was identified. This line was crossed (Fig. 5) to a line (AGS 406) with good agronomic attributes but with poor germination ability. The germination ability of both these lines were determined after storing the seeds for about 6 months from harvest. In the F_2 generation of the above cross, germination, pubescence, and days to first flowering (not presented) were recorded. A Chi square analysis of the population data on the presence of pubescence was performed.

Results and Discussion

The analysis of variance revealed significant (P <0.0001) variation for percentage of germination, seed size and seed weight. GC-84501-32-1 showed the highest germination percentage at 96% (Fig. 6), while AGS 330 showed the lowest at 0% (Fig. 7; Fig. 8). GC-84501-32-1 recorded the lowest seed size while AGS 456 W recorded the largest seed size (Fig. 9). GC-84501-32-1 also had the lowest seed weight while AGS 404 and 406 had the highest seed weights (Fig. 10).

The correlation analysis (Pearson Correlation Coefficients) showed that there was a weak negative relationship between both seed size (-0.15) and seed weight (-0.17) to percentage of germination. These results can be compared to a recent study that found that medium seeds had higher germination percentage than that for large and small seed sizes (Rezapou *et al*, 2013). Another study found that medium seed weights had higher germination rates (Adebisi *et al*, 2012). The analysis in this study showed that there was strong positive relationship (+0.64) between seed size and seed weight.

Out of 70 seeds sown of the F_2 segregating population from the cross between AGS 406 and GC-84501-32-1, only 46 germinated. Presence of pubescence on the stem was recorded. Out of 46 plants, 32 were glabrous and 14 were pubescent (Fig. 11 and Fig. 12). The Chi Square analysis showed that glabrous is a dominant trait over pubescence and is controlled by a single gene (Nagai and Saito, 1923).

Fig.2. The different soybean lines utilized for the germination experiment.

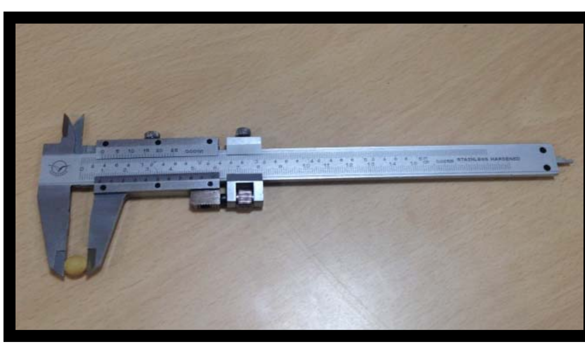


Fig.3. Vernier caliper used for measuring the seed size. Fig. 4. The layout of the germination experiment.

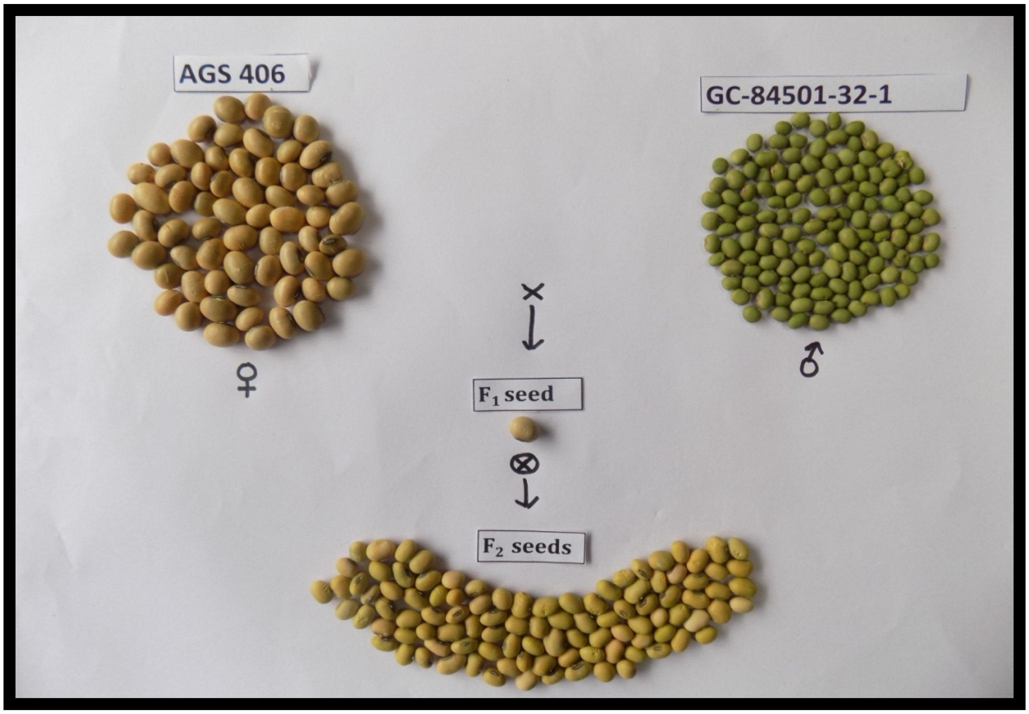


Fig. 5. Seeds of the different generations from the cross between line with high germination ability and the line with larger seed.

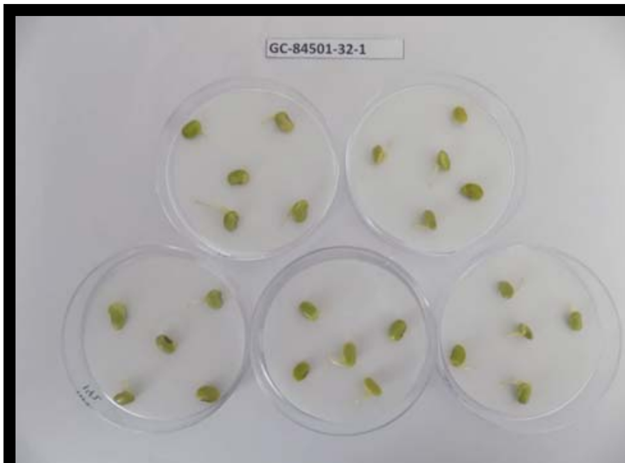


Fig. 6. Excellent germination in GC 84501-32-1. Fig. 7. AGS 330 showing poor germination.

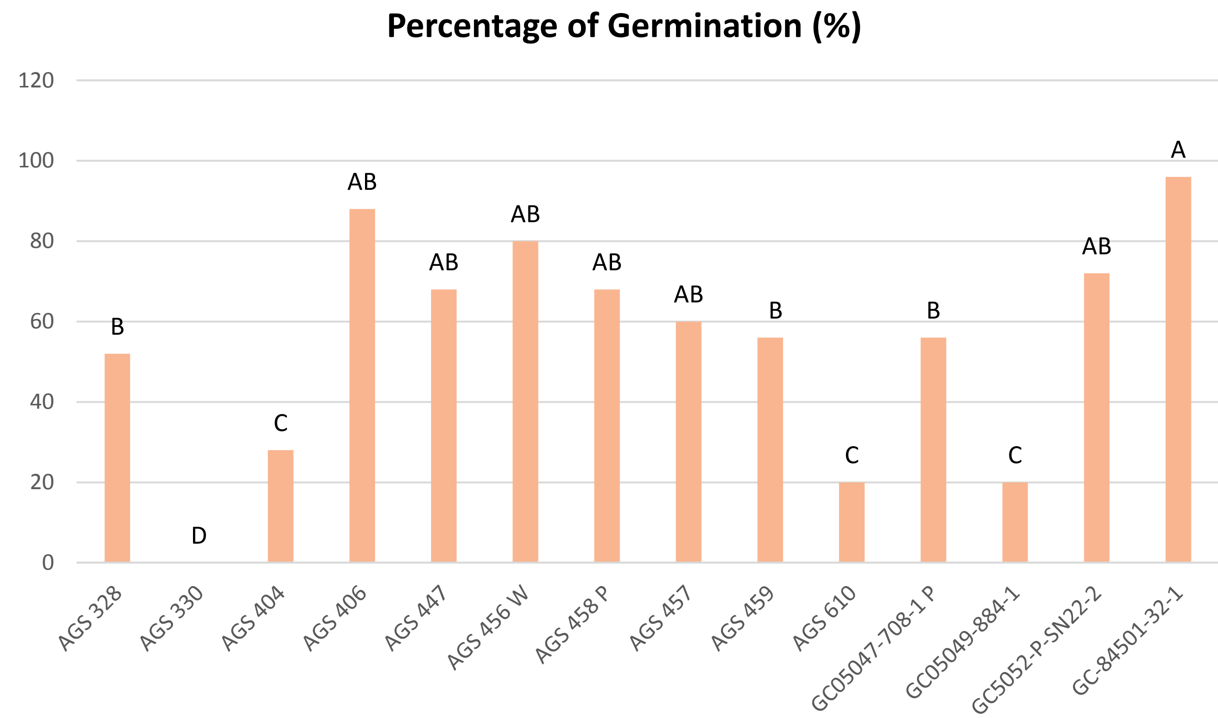


Fig.8. Variation for percentage of germination in soybean lines. (Means with the same letter are not significantly)

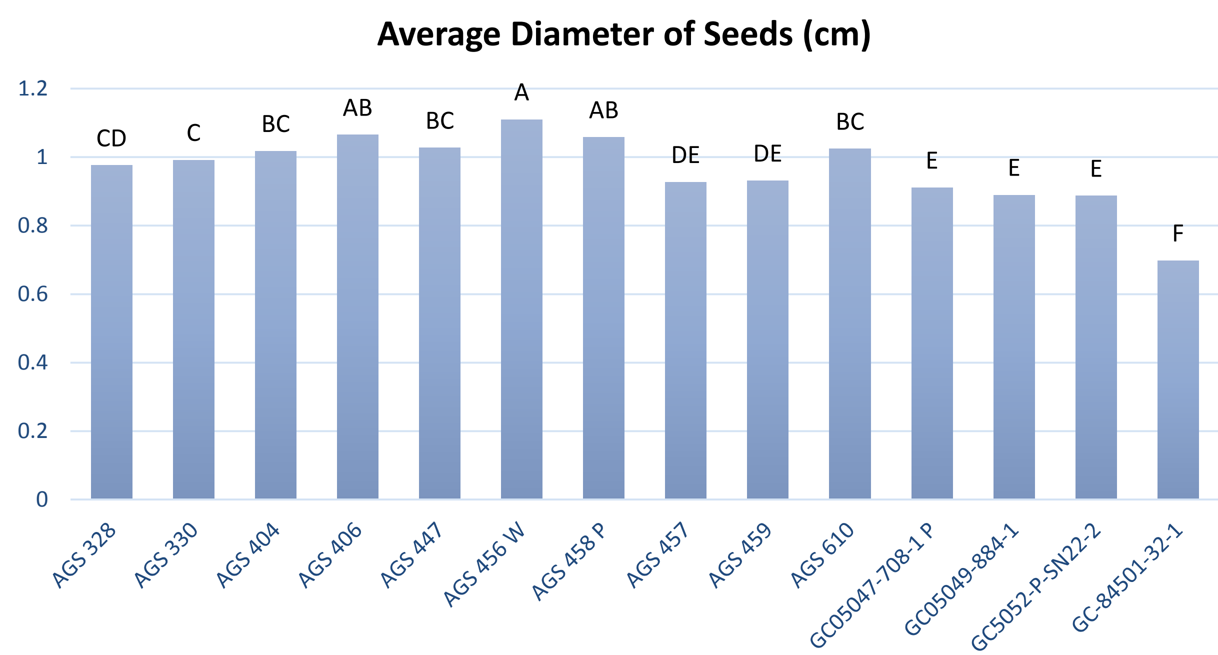


Fig.9. Variation for seed size in soybean lines. (Means with the same letter are not significantly)

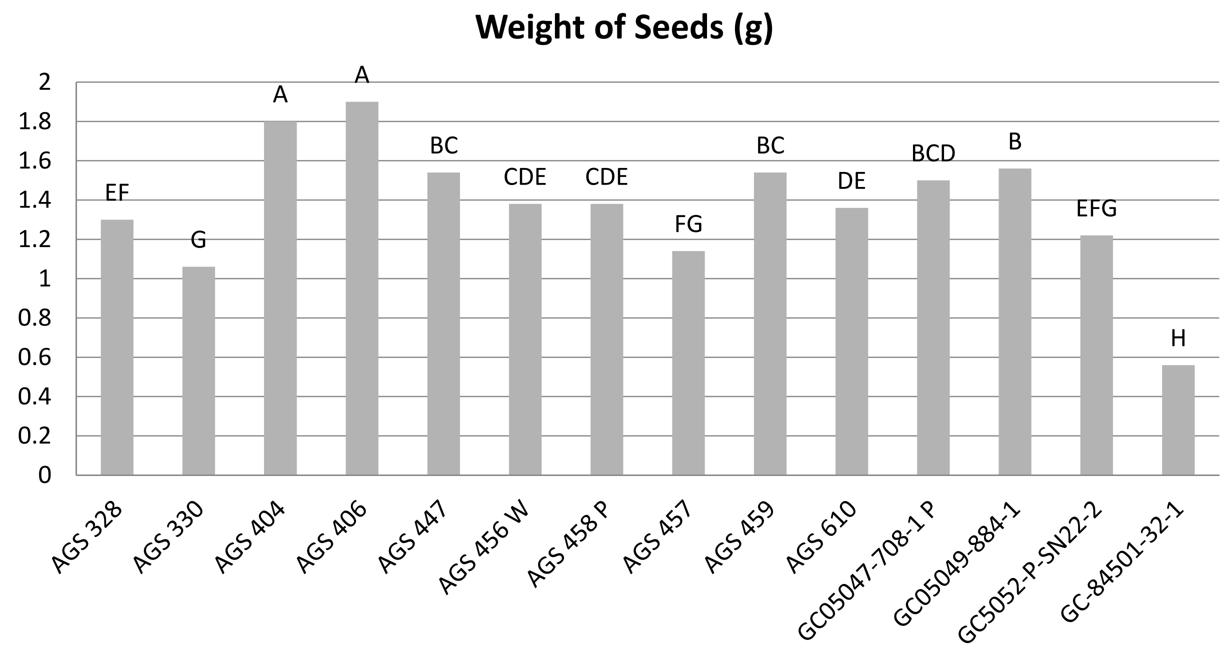


Fig.10. Variation for seed weight in the soybean lines. (Means with the same letter are not significantly)

Fig.11. Pubescence Pods of Vegetable Soybean Fig. 12. Glabrous Pods of Vegetable Soybean

Conclusion

Line GC-84501-32-1 could be further tested for its agronomic properties and also could be utilized as donor parent for improving germination ability in vegetable soybean. A chemical composition analysis of the seeds of this line may be undertaken in order to identify any chemical compound associated with improved germination ability. Data collected from the F_2 generation population confirmed the true nature of the cross between the two lines, AGS 406 and GC-84501-32-1. The correlation study indicated that the approach of crossing between the two lines would be required to break the negative relationship between the seed traits and the germination ability. Seeds collected from the F_2 population may be grown on a plant to progeny basis and germination ability assessed in the F_3 generation for further selection. Vegetable soybean varieties with high germination ability will ensure a good crop stand for vegetable soybean farmers.

References:

Adebisi M., Salau A., Okesola L., Porbeni J., Esuruoso A., Oyekale K. (2012). Influence of Different Seed Size Germination, Seed Emergence and Seed Yield Characteristics in Tropical Soybean (*Glycine max* L. Merril). *International Journal of Agriculture Research.*

Nagai, I., Saito, S. (1923) Linked factors in soybean. *Japanese Jour. of Bot*. 1: 121-136.

Rezapour R., Kazemi-arbat H., Yarnia M., Zafarani-Moatta P. (2013). Effect of seed size on germination and seed vigor of two soybean (*Glycine max* L.) cultivars. *International Research Journal of Applied and Basic Sciences.* Vol 4 (11), 3396-3401. Science Explorer Publications. Tabriz, Iran.

Shanmugasundaram, S., Nair, Ramakrishnan M., Yan, Miao-Rong., Palada, Manuel C. (2015). "Vegetable Soybean (Edamame)." Handbook of Vegetables. Vol. 3. Houston: Studium LLC, 521-549.pp.

Personal Remarks: My Journey

Both parents having an occupation in the science field, I have grown up to appreciate the world around me and what it can offer with a questioning nature. As a little child I began to mix together various things to see what concoctions I could make. By fourth grade, I started actual experimentation with yeast at varying temperatures. By the time I reached high school, I began to work in a lab using lab equipment to conduct my experiments. Through a few agriculture classes offered at my high school through the program FFA (Future Farmers of America) I found that I really enjoyed working in the field of Food Science. Through this same inspiring FFA classes, my teacher and advisor introduced me to the World Food Prize Foundation as an opportunity. I never knew it would have quite the impact to change my life like it has.

My two older sisters had already been involved in this program. I can remember being eight years old and my mother and oldest sister, Hannah, coming homing and announcing to the family that she would be gone for two months to go to China. Not fully understanding, all that really meant was that I wasn't going to have to be around my sister for two months. When Hannah cam e back from China, it was like she was a different person. Her whole view changed including her outlook on her future. A few years later my second older sister, Sarah, received the news she would be traveling to Taiwan with the same internship. She, like my other sister, came back so excited and inspired. I remember deciding then that I wanted to have similar experiences as they described.

Eager to get involved I planned to go on a mission trip to Haiti. While there poverty shock hit me hard. I saw small villages that you could measure wealth by whether they had clothes or not. I remember reading about poverty and seeing heart wrenching pictures, but nothing compared to actually experiencing it. I was humbled. There started sparks of passion in me to aid people in any way I can.

It was in Haiti that I found my inspiration to write a paper for the World Food Prize Foundation on education issues. That began my journey that took me farther then I will ever know. A few months later I found myself sitting enthusiastically among other bright young students immersed with prestigious world leaders talking about agriculture and global problems. Even faced with such challenges of world hunger and poverty, our spirits rose to tackle them and become the next Norm.

Inspiration running high, I applied for the Borlaug-Ruan internship like my sisters before me. I made it through the various stages of selection to find out that I had been selected to work for the AVRDC- the World Vegetable Center, South Asia in Hyderabad, India. I was jubilant to be selected for an organization that is so aligned with my passions of alleviated world hunger through agriculture and food science.

This Borlaug-Ruan International Internship has helped me to gain a new perspective on attacking global poverty. It has shown me just how complicated poverty can be. There are no easy solutions, but through the blood, sweat, and tears and the many warriors bonded together we can make a difference and save human lives.

I can say that I am so thankful for my experiences and all the people that I have met here. I will never forget my time here among such motivating and influential people brought together by a common goal to help people and end world hunger. They turned my kindling sparks of passion into a zealous blazing fire. I love my Indian family and cannot thank them enough for everything they have done for me. They will always have a lasting impact on me and a special place in my heart.

Thank you again to everyone who has made this experience possible. I hope to continue to have them along with my fight against hunger and service to people.

Sincerely

~Mariah

Photos: Work at AVRDC

Sowing mungbean seeds in germination paper to help with a mungbean sprout experiment ►

◄ Emasculating a vegetable soybean flower bud with prospects to breed

Sowing Vegetable soybean seeds in field ►

◄ Identifying Vegetable soybean flower bud stage

◄ My supervisor and I with the different varieties of legumes

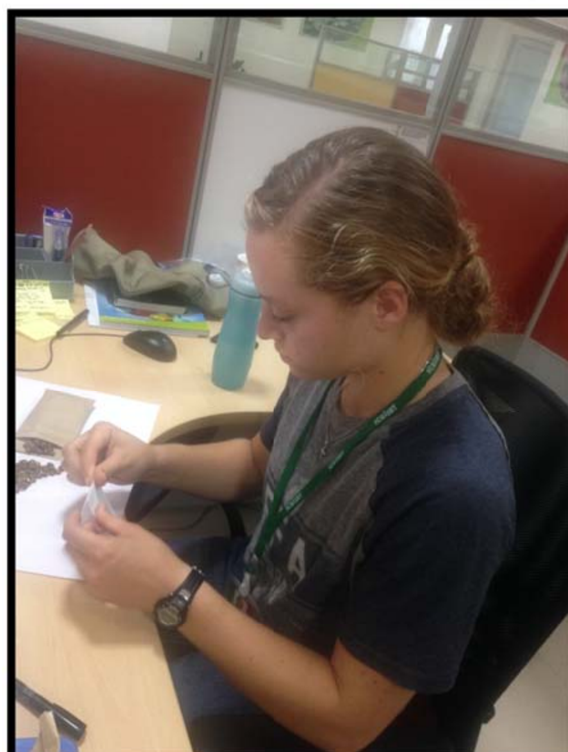
Recording data from field experiment ►

◄ My mentor and I placing vegetable soybeans in petri dishes for experiment

Recording observations from glasshouse breeding population of vegetable soybean ►

My roommate and fellow Borlaug-Ruan Intern, Precious, and I at the ICRISAT entrance

Sorting, Selecting and counting vegetable soybean seeds for experimentation ►



◄ Plant Quarantine where imported varieties of vegetable soybean are first grown

Presenting my project to AVRDC and friends (above left), AVRDC regional director Warwick and Supervisor Dr. Ram presenting me gifts after my presentation (above right), All of AVRDC staff and friends (below)

Photos: Cultural







References:

"500 Million Vegetarians In India." (2013). Vegetariansconz. Web. 5 Aug. 2015. <http://www.vegetarians.co.nz/articles/500-million-vegetarians-in-india/>.

[AOSA] Association of Official Seed Analysts (2009). AOSA rules for testing seeds. P. 6-3. Ithaca (NY):

Becker, H. (1993). *Pflanzenzucbtung. Stuttgart: Verlag Eugen Ulmer.*

Bellaloui, N., Reddy, K., Bruns, A., Gillen, A., Mengistu, A., Zobiole, L., Fisher, D., Abbas, H., Zablotowicz, R., Kremer, R. (2011). Soybean seed composition and quality: Interactions of environment, genotype, and management practices. Soybean: Cultivation, Uses and Nutrition.

Barton, L.V. (1943). Effect of moisture fluctuations on the viability of seeds in storage. Boyce Thompson Inst. 13:35-45

Bernal-Lugo, C., Leopold, A.C. (1995). Seed stability during storage: Raffinose content and seed glassy state. *Seed Sci*. Res. 5:75-80.

Bernal-Lugo, I., Leopold, A.C. (1998). The dynamics of seed mortality. *J. Exp. Bot.* 49: 1455-1461.

Bewley, J.D., Black, M. (1994). Seeds: physiology of development and germination. 2nd ed. Plenum Press, NY

Boopathy, G., Maitra, S., Bhaskar, S., Shanti, R., Hansraj. (2014). Millennium Development Goals India Country Report 2014. Social Statistics Division Ministry of Statistics and Programme Implementation Government of India <www.mospi.nic.in>

Burris, J.S. (1980). Maintenance of soybean seed quality in storage as influenced by moisture and genotype. *Iowa State J. Res.* 54: 377-389

Byrd, H.W., Delouche. J.C. (1971). Deterioration of soybean seed in storage. Proc. Assoc. Off. Seed Anal. 61:41-57.

Cherry, J. H., Bishop, L., Hasegawa, P M., Heffler, H. R. (1985). Differences in the fatty acid composition of soybean seed produced in northern and southern areas of the U.S.A. *Phytochemistry, 24, 237-241.*

Clemente, T.E., Cahoon, E.B. (2009). Soybean oil: genetic approaches for modification of functionality and total content. *Plant physiol*. 151:1030-1040.

Copeland, L.O., McDonald, M.B. (2001). Principles of seed science and technology. 4th ed.Kluwer Academic Publishers. Norwell, Massachusetts

Delgado C., Narrod C., Tiongco M. (2003). Policy, Technical, and Environmental Determinants and Implications of the Scaling-Up of Livestock Production in Four Fast-Growing Developing Countries: A Synthesis. 2.3 Growth and Concentration in India[6]. Web. <http://www.fao.org/wairdocs/lead/x6170e/x6170e09.htm>.

Delouche, J.C. (1968). Precepts of seed storage. Mississippi Short Course Proceed. pp 97-123.

Delouche, J.C., Caldwell, W.P. (1960). Seed vigor and vigor tests. Proc.Assoc. Offic. Seed Anal. 50:124-129.

FAO, (2013). Food and Agriculture Organization of the United Nations

FAOSTAT, (2013). Food and Agriculture Organization of the United Nations

GAIN, (2014). Global Alliance for Improved Nutrition Harrington, J.F. 1959. The value of moisture resistant containers in vegetable seed packaging. Cal. Agr. Exp. Stat. Bull. 792: 1-23

Harrington, J.F. (1973). Biochemical basis of seed longevity. *Seed Sci. & Tech.* 1:453-461.

Hartwig, E. E., Kuo, T. M. & Kenty, M. M. (1997). Seed protein and its relationship to soluble sugars in soybean. *Crop Sci., 37*, 770-773.

Haussmann, B., Rarzies, H., Susic, Z., Miedaner, T. (2003). Plant genetic resources in crop improvement. Institute of Plant Breeding, Seed Science, and Population Genetics and State Plant Breeding Institute, University of Hohenheim, 70593 Stuttgart, Germany

Hepperly, P.R., Sinclair. J.B. (1982). Relationships among *Cercospora kikuchii*, other seed mycoflora, and germination of soybeans in Puerto Rico and Illinois. *Plant Dis.* 65:130-131.

Hou, A., Chen, P., Alloatti, J., Li, D., Mozzoni, L., Zhang, B., Shi, A. (2009). Genetic variability of seed sugar content in worldwide soybean Germplasm collections. *Crop Sci., 49*, 903-912

Hou, C.J. (2004). Effects of temperature, seed water content and oxygen on the storage longevity of vegetable soybean and black soybean seeds. Department of Agronomy, National Chiayi University, Chiayi, Taiwan

Hymowitz, T., Collins, F. I. (1974). Variability of sugar content of seed of *Glycine max* (L.) Merr. and *G. soja* Serb. and Zucco. *Agron. J., 64*, 239-240.

Hymowitz, T., Collins, F. I., Panczar, J., Walker, W. M. (1972). Relationship between the content of oil, protein, and sugar in soybean seed. *Agron. J., 64*, 613-616.

"India Population (LIVE)." Population of India (2014). Web. 13 Aug. 2015. <http://www.worldometers.info/world-population/india-population/>.

Integrated Crop Management, University of Iowa, USA, http;//www.ipm.iastate.edu/ipm/icm/2003/10-6-2003/charcoalrot.htm Verified (4/14/2010).

Johnson, R.R., Wax. L.M. (1978). Relationship of soybean germination and vigor tests to field performance. Agronomy J. 70:273-278

Kaur, K. (2014). Anaemia 'a silent killer' among women in India: Present scenario. *European Journal of Zoological Research.*

Krittigamas, N., Vearasilp, S., Thanapornpoonpong, S., Suriyong, S., Pa-oblek, S., Pawelzik. E. (2001). Investigation of post-harvest soybean seed storability after passing the different steps of processing. In: M. Becker, editor, Deutscher Tropentag 2001 Conference on International Agricultural Research for Development, Bonn, Germany. 9–11 Oct. 2001. Margraf Publishers – Scientific Books, Weikersheim, Germany. ftp://ftp.gwdg.de/pub/tropentag/proceedings/2001/full%20papers/17-post%20harvest/17_2_Krittigamas1. pdf (verified 19 Feb. 2013).

Kulik, M.M., Schoen, J.F. (1981). Effect of seed borne *Diaporthe phaseolorum* var. *sojae* on germination, emergence, and vigor of soybean seedlings. *Phytopathol*. 71:544-547.

LeVan, N.A., Goggi, A.S., Mullen, R. (2008). Improving the reproducibility of soybean standard germination test. *Crop Sci*. 48: 1933-1940.

Lisker, N., Ben-Efraim, A., Henis, Y. (1985). Fungi growing on stored soybeans and their significance in lipid breakdown. *Ann. Applied Biol.* 107:117-126.

"Malnutrition." Health Issues India. 23 Jan. 2012. Web. 13 Aug. 2015. <http://www.healthissuesindia.com/malnutrition/>.

Mausda, R. (1991). Quality Requirement and Improvement of Vegetable Soybean. In: Vegetable Soybean, Research Needs for Production and Quality Improvement. National Food Research Institute, Ministry of Agriculture, Forest and Fisheries, Japan. 92-102.

McGee, D.C. (1995). Epidemiological approach to disease management through seed technology. Annu. Rev. *Phytopathol*. 33:445-466.

Mbofung, G. Y. (2012). "Effects of maturity group, seed composition and storage conditions on the quality and storability of soybean (*Glycine max* L. Merrill) seed" (2012). Graduate Theses and Dissertations. Iowa State University Paper 12596.

Morales, A., Kokini, J.L. (1997). Glass transition of soy globulins using differential scanning calorimetry and mechanical spectrometry. *Biotechnol. Prog.* 13:624-629.

Nattasik, K.S., Vearasilp, A., Thanapornpoonpong, S., Suriyong, S., Pa-oblek,S., Pawelzik, E. (2001). Investigation of post-harvest soybean seed storability after passing the different steps of processing. Conference on International Agric. Res. Dev. Deutscher Tropentag.

Nightingale, M.J., Marchylo, B.A., Clear, R.M., Dexter, J.E., Preston. K.R. (1999). Fusarium head blight: Effect of fungal proteases on wheat storage proteins. *Cereal chem.* 76:150-158.

Roy, K. W., Baird, R.E., Abney, T.S. (2000). A review of soybean (*Glycine max*) seed, pod, and flower mycofloras in North America, with methods and a key for identification of selected fungi. *Mycopathologia* 150:15-27.

Sankar R., van den Briel T. (2014). Prospects for better nutrition in India. Web. <http://www.ncbi.nlm.nih.gov/pubmed/25384721>

Schnebly, C. W., Fehr, W. R., Welke, G. A., Hammond, E. G., Duvick, D. N. (1994). Inheritance of reduced and elevated palmitate in mutant lines of soybean. *Crop Sci., 34, 829-833.*

Schortt, B.J., Sinclair, J.B., Helm, C.G., Jeffords, M.R., Kogan. M. (1981). Soybean seed quality losses associated with bean leaf beetles and *Alternaria tenuissima*. *Phytopathol.* 72:615-618.

Sharma, S., Kaur, A., Bansal, A., Gill, B. (2011). Positional effects on soybean composition during storage. Association of Food Scientists & Technologists, India

Singh, B., Gunasena, P. (1974). Effect of Storage Temperature on Seed Germination in Soybean *Glycine max* (L.) Merr.

Smith, J.R., Mengistu. A. (2010). Seed quality of high-germinability soybean lines after six months of ambient storage. Crop Science Society of America. Abstract

"Southeast Asia: India." (2010) Central Intelligence Agency. Central Intelligence Agency. Web. 5 Aug. 2015. <https://www.cia.gov/library/publications/the-world-factbook/geos/in.html>.

Spears, J.F., Tekrony, D.M., Egli. D.B. (1997). Temperature during seed filling and soybean seed germination and vigor. *Seed Sci. Technol*. 25:233-244.

Shanmugasundaram S., Nair, Ramakrishnan M., Yan, Miao-Rong., Palada, Manuel C. (2015). "Vegetable Soybean (Edamame)." Handbook of Vegetables. Vol. 3. Houston: Studium LLC, 521-549. pp.

Sun, Q., Wang, J., Sun, B. (2007). Advances on seed vigor physiology and genetic mechanisms. *Agric. Sci.* China 6:1060-1066.

Tang, S., Tekrony, D.M., Egli, D.B., Cornelius, P.L. (2000). An alternative model to predict corn seed deterioration during storage. *Crop Sci*. 40:463-470.

Thomas, W. (1979). Seed viability in relation to storage conditions in varieties of soybean. Mysore J *Agric Sci* 14:271-273

USDA National Nutrient Database for Standard References, (2011). Release 27.

Vertucci, C.W., Roos, E.E. (1990). Theoretical basis of protocols for seed storage. *Plant Physiol*. 94:1019-1023.

Vertucci, C.W., Roos, E.E. (1993). Theoretical basis of protocols for seed storage II. The influence of temperature on optimal moisture levels. *Seed Sci*. Res. 3:201-213.

Vieira, R.D., Tekrony, D.M., Egli, D.B., Rucker, M. (2001). Electrical conductivity of soybean seeds after storage in several environments. *Seed Sci*. Technol. 29:599-608.

Wang, X.G., Chen, J.G., Zhu, Y.S., Li. L.S. (2000). Studies on the vigor changes during seed maturation of Russian wiltype. *Acta Agresitia Sinica* 8:306-311.

WHO, (2000) Geneva, World Health Organization.

Wilson, R.F., Rinne, R.W. (1974). Phospholipids in the developing soybean seed. *Plant Physiol*. 54:744-747.

Yu, S.B., Chen, W.Z., Xu, C.G. (1999). Genotypic variation of seed vigor in rice. *Seed* 2:24-26.