

Michigan State University



The Story of Bean Breeding

White paper prepared for BeanCAP & PBG Works
on the topic of dry bean production and breeding
research in the U.S.

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Crops of the Americas: The crops depicted in the stamps—corn, chili peppers, beans, squashes, and sunflowers—had been cultivated in the Americas for centuries when Europeans first arrived in the New World. The stamp art includes ears of corn (*Zea mays*); red and green chilies (*Capsicum annuum*); lima beans (*Phaseolus lunatus*), scarlet runner beans (*P. coccineus*), and pintos and other common beans (*P. vulgaris*) described on page 3; various squashes (*Cucurbita* spp.); and a sunflower and seeds (*Helianthus annuus*). Courtesy of the U.S. Postal Service.

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On the Cover: Dry Bean Nursery Trial Plots planted near Saginaw, MI

PREFACE

The white paper entitled “The Story of Bean Breeding” was written to provide an overview of the science of bean breeding from historical, even ancestral times, to the modern day use of molecular tools and genomics to develop new bean varieties.

The paper chronicles an overview of the breeding process, time-line, priority setting, objectives, different traits, germplasm sources, public and private breeding programs and researchers. The information presented represents the work and ideas of the author who has spent over 40 years conducting research on various aspects of bean improvement at different institutions. To provide as complete a picture of all aspects of breeding and genetics, the author has made extensive use of web sites where more detailed information is available on specific topics. These sites are listed at the end of the paper. One of the best sites to describe plant breeding is the short video “Plant Breeding: A modern career” prepared by plant breeders at Michigan State University.

<http://www.hrt.msu.edu/pbgp/Links/plantbreedingintro.html>



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INTRODUCTION AND HISTORICAL BACKGROUND OF DRY BEAN GENETICS AND AGRONOMY



Fig 1. Diversity in plant type, seed and pod type, and flower color in *Phaseolus*. Photo courtesy of M. Nenno

Description of bean species

The common bean (*Phaseolus vulgaris*) is a member of the legume family Fabaceae which defines it as a member of a plant family that produces pods that carry a nutrient dense high protein seed. All legumes (grain or forage) fix atmospheric nitrogen in a symbiotic relationship with *Rhizobium* bacteria in the soil. Beans are related to soybean (that originates in China) having diverged some 18 million years ago (mya), but they diverged more recently (8 mya) from the cowpea (southern pea). *Phaseolus vulgaris* is recognized by a large number of common names that include all dry edible bean seed types – pinto, kidney, navy beans and different names for garden beans such as runner beans, string beans, half-runners, snap beans, French, and haricot beans. The common bean is a member of the Genus *Phaseolus* (Fig. 1) for which there are five cultivated species but the common bean is the most widely grown and the most economically important worldwide. The other four types: the scarlet runner bean (*P. coccineus*, known for its red/scarlet flower color); year-bean (*P. dumosus*, aka *P. polyanthus*, just named a species in 1995);

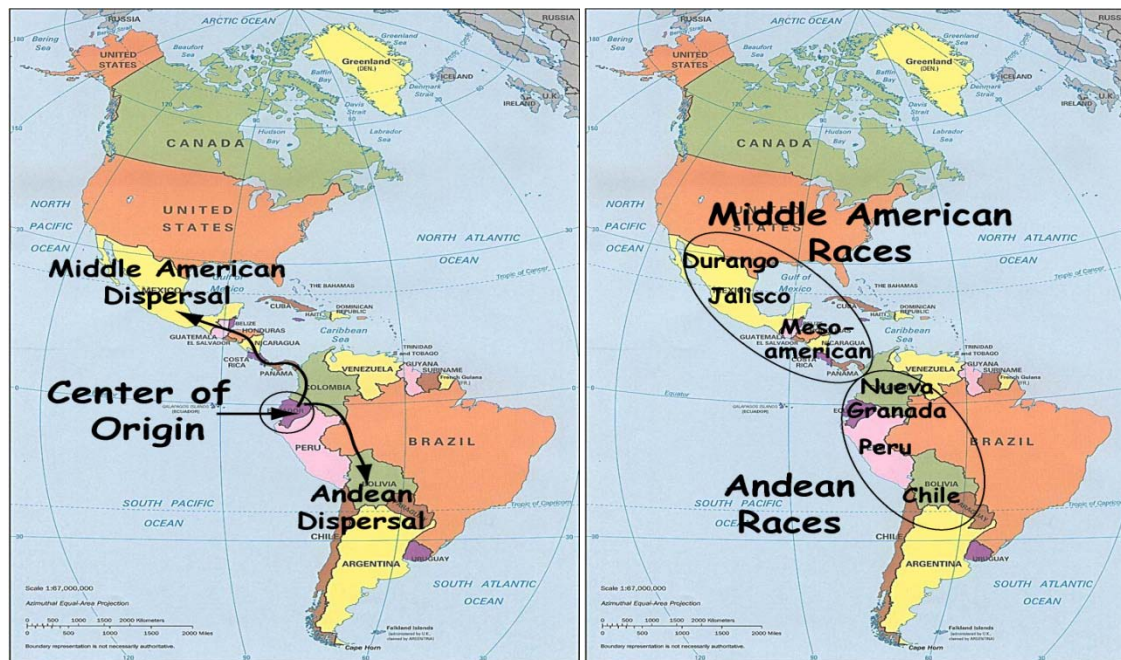
teparty bean (*P. acutifolius*); and lima bean (*P. lunatus*). They are listed in order of genetic similarity to the common bean. These four cultivated species have similar origins in the Americas, tepary found in NW Mexico and southern Arizona, scarlet runner and year-beans found in Southern Mexico and the highlands of Guatemala and the lima bean mirrors the common bean in that it has two centers of origin: one in Andean region and the other in Middle America. In addition to the five cultivated bean species there are over 50 species of wild beans in existence today. Since these are distinct genetic species, common bean can only be crossed with a few wild species and a few of the cultivated species. Common bean can be crossed with *P. coccineus* and *P. dumosus* in one direction (male only); and successful crosses with tepary bean require an embryo rescue step to produce viable hybrids. Genes for disease resistance have been successfully moved from tepary to common bean. No successful hybrids have been produced between lima and common bean. Common bean has a wide diversity of seed and pod types, plant habits from bush to climbing bean, range of maturities, photoperiod sensitivity and neutrality, adaptation zones, wide range of disease and stress resistances and different nutritional quality components. This genetic variability is used by breeders to further enhance the crop.

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Major gene pools (Middle American & Andean)

The common bean is a new world crop having its origins in South America. The ancestral wild bean is believed to have originated in Southern Ecuador/ Northern Peru. From there it moved southwards as far as Salta in Northern Argentina and northwards to Chihuahua, Mexico. Wild beans can be found in the same geographic range today. The basis of the origin is based on DNA analysis that shows that the simplest DNA structures exist in wild beans from these regions of Ecuador and Peru. Analysis of wild beans from other regions suggests more genetic organization suggesting more recent origins. Wild beans were domesticated independently throughout the geographic range from Argentina to Mexico. Those domesticated in the Andean regions from Ecuador south are known as belonging to the Andean gene pool, whereas those domesticated from Colombia northwards belong to the Middle American gene pool. There appears to have been limited domestication events in the Andean gene pool (based on DNA analysis) resulting in less genetic diversity in this gene pool. In contrast multiple domestication events are recorded in the Middle American gene pool and there is greater genetic diversity in this pool. One obvious contrast between the gene pools is the seed size of the beans. Both wild and cultivated beans from the Andean gene pool are larger than the corresponding beans from the Middle American gene pool.

(Figs. 2 and 3 below display the ancestral origin and movement of wild bean; and the races of domesticated common bean within the two major gene pools; Figs courtesy of P. McClean)



The gene pools are further subdivided into races based on agro-ecological adaptation. These races correspond to the broad commercial seed classes that are grown in the U.S. In the Andean gene pool, Nueva Granada race includes large seeded kidney beans, bush cranberry beans and most snap beans. Race Peru includes the yellow beans (Mayacoba and Canario) and Race Chile includes the vine cranberry beans and an array of types unique to Chile (Coscarron and Tortula).

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Within the Middle American gene pool, the small seeded navy and black beans belong to the Mesoamerican race; pinto and great northern beans belong to the Durango Race; and small red and pink beans belong to Jalisco Race (Fig 3). A fourth race unique to Guatemala includes only climbing beans types. Breeders can freely cross between races and/or gene pools although inter gene pool crosses have had limited success. Beans were likely domesticated before pottery was widely available and the original pop bean (Nuñas) that were cooked on hot stones in fires still exist in highland Peru today. Beans domesticated in the last 5,000 years were moved through trade northwards to the U.S., pinto and great northern entered the central U.S. and were moved by native peoples (Mandan Indians) as far north as the province of Alberta; navy and black beans were carried from the Caribbean up the east coast to the Great Lakes region; and the large seeded kidney beans were moved as part of the slave trade by Europeans to Europe and on to East Africa (secondary center of domestication) and returned to the U.S. east coast as heirloom varieties introduced by the early settlers. The navy bean was probably christened by Commodore Perry during the War of 1812 (Lake Erie) as the dry bean was valued as a high protein food that was easily stored and transported. These bean types were first grown by native people around the Great Lakes and valued by the early settlers as a high protein food source adapted to the region.

Michigan Dry Bean Classes



Black beans



Navy beans



Light Red Kidney beans



Dark Red Kidney beans



Cranberry beans



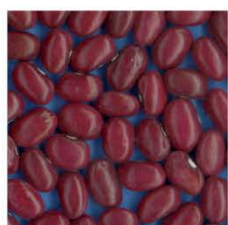
Great Northern beans



Pinto beans



Yellow eye beans



Small Red beans



White Kidney beans



Pink beans



Soldier beans

Fig 4. Diversity of bean seed size, shape and color in major commercial classes grown in the U.S. Photo courtesy of L. Copeland.

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Agronomic characteristics and limitations

The dry bean is recognized as a short season crop (under 100 days) that despite unfavorable climatic or edaphic factors will always produce seed. In contrast, maturities ranging from 4-6 months are common among climbing beans grown in highland Andean regions. Beans (non-climbing) are therefore generally produced in those regions where the growing season is limited in northern production areas or at higher elevations where long season crops are at a disadvantage. Beans do not thrive in hot humid environments. Throughout modern agricultural history beans have been undervalued in relation to other crops and in most countries bean production has been pushed on to less favorable soils in either drought or frost prone regions that limit production. The best land in the U.S. and other major bean producing countries such as Mexico and Brazil is used to produce corn and soybean, so bean yields will never be competitive on less favorable land. With the exception of heirloom beans cultivated in gardens, all commercial beans grown in the U.S. are either bush types, some very determinate in habit like kidney beans or others have an upright short vine similar to soybean that permits direct harvest, while others produce a long prostrate vine that is very productive under drier conditions west of the continental divide where rainfall is not a threat during harvest. Yields in beans are limited in part by the short growing season (compared with soybean), susceptibility to an array of viral, fungal and bacterial diseases, and insect pests, more prone to stress as recovery time is limited, nutrient deficiencies, and the limited investment in research to improve the crop. Private plant breeding focuses largely on snap beans (higher return on investment in seed sales) as efforts in dry bean breeding are diluted across ten different commercial classes with specific quality attributes that require individual attention. The limited acreage (~1.5m acres) base distributed across ten seed classes does not favor private sector investment. Beans are a non-GM (Genetically Modified) crop and those in production feel that this further limits their productivity and grower acceptance/profitability. Making comparisons with breeding progress in the major crops in the U.S. is not realistic so comparisons are better made with other grain legume (pulse) crops such as pea, lentil and chickpea as they suffer from many of the same physiological and economic limitations as do beans.

Indigenous and commercial production

Commercial bean production in the U.S. is largely limited to short season northern production areas of the Great Lakes and Upper Midwest and higher altitude locations west of the continental divide. The shorter growing season of beans allows them to fit well into the cropping systems in these northern production areas. Production costs depend on location/state (land costs) and the need for irrigation. Bean production east of the Mississippi is largely rainfed. In Michigan less than 5% of the bean production is irrigated. In the east, irrigated production regions are restricted to regions of Michigan, Wisconsin, and Minnesota on drought prone sandy soils where kidney beans thrive. Production west of the Mississippi is essentially all irrigated with exception of limited pinto acreage in the four-corner region of Colorado and as a result production costs are higher. Production costs in the Central Valley of California have largely driven commercial bean production from that state and have converted Idaho into a major seed producer of snap and dry beans. Yields are lower in the eastern U.S. due to the vagaries in rainfall distribution while the highest yields are found in the Great Basin of Washington where adequate sunshine, irrigation and the absence of most major foliar disease problems favor high productivity. Traditionally,

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individual states were known as producers of a single commercial bean class [NY-kidney, black; MI-navy, cranberry; CO-pinto; NE-great northern; ID-pinto, pink; WA-small red; CA-pink, kidney], but most states have diversified to meet market needs. North Dakota is the leading production state but as corn and soybean production expands in that state, beans are being pushed out of the productive soils of the eastern Red River Valley into the shallower soils and shorter season regions in the center of the state repeating similar trends that have occurred in MI, NY and CA. Only favorable grower prices will stabilize or reverse such negative trends.

Beans are planted as a row crop in all states, with planting in early June and harvest from mid to end of September depending on location and season. Seed is slurry treated with a combination of insecticide, fungicide and bactericide to control pests. Weeds are controlled mainly through use of pre and post applied herbicides, and producers are shrinking row widths (30" - 22" - 15") to maximize productivity. The threat from diseases such as white mold increases under these more intensive management systems. In certain seasons a fungicide treatment is required to control white mold. The crop requires modest level of fertility (50 pounds actual N/acre) applied at planting and usually one insecticide spray to control leafhoppers. There is increasing interest in all production areas to reduce costs by direct harvesting the crop which allows growers to harvest more acres in a specific time span, reducing the need for specialized equipment and tractor use, and permits growers to increase their bean acreage. Direct harvest produces a 'cleaner' bean (less soil) but seed coat checks can increase unless care is taken to prevent damage during harvest in dry years. Larger seeded kidney and cranberry beans still require specialized harvest equipment to maintain seed quality.

Production data by class and region

Bean production regions have seen major changes over the years for the reasons discussed above. The most current data on production by bean class is available on US Dry Bean Council web site http://www.usdrybeans.com/home/default_usr.aspx and the most up to date production figures are available through the Economic Research Service, USDA, 2010. *Vegetables and Melons Outlook/VGS-337/February 25, 2010.* <http://www.ers.usda.gov/Briefing/drybeans/PDFs/DBnOutlook.pdf>. <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1394>.

Directory of Bean Scientists

The majority of public bean researchers in the U.S. participate in the W1150 Regional Project entitled: "Exotic Germplasm Conversion and Breeding Common Bean (*Phaseolus vulgaris* L.) for Resistance to Abiotic and Biotic Stresses and to Enhance Nutritional Value" The project fosters scientific collaborations, and researchers meet annually which provides a forum to share new scientific findings on different aspects of bean improvement. The project was selected as the best project in the western region in 2009.

<http://nimss.umd.edu/homepages/member.cfm?trackID=7076>

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REVIEW OF THE FUNDAMENTALS OF PLANT BREEDING

Genetic knowledge of the traits being improved is of major importance to bean breeders. Breeders need to know the inheritance of a trait in order to choose the most effective breeding method to enhance that trait. Traits are either qualitatively (controlled by single or few genes) or quantitatively (multigenic) inherited. Examples of a qualitative trait would be flower color where purple flower is controlled by single dominant gene and white color is controlled by a single recessive gene. Flower color is easy to select for and follows simple Mendelian segregation of 3 purple: 1 white.

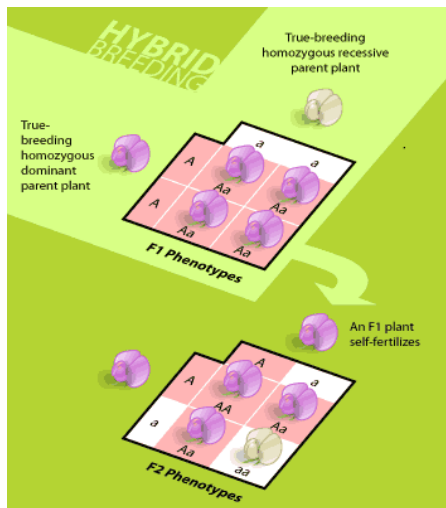


Fig 5. Two pure breeding beans can be hybridized to create cross progeny which exhibit the desired phenotypes of both parent species. Here a recessive white flowered breed is crossed with a dominant pink flowered breed which contains a gene enabling it to grow in colder conditions. The desired phenotype is a white flower which can grow in the colder conditions. The offspring of the hybridization of the pure species is followed by selection and breeding to generate the desired plant. Courtesy J. Pighin, Science Creative Quarterly, 2009.

Breeders can make fast progress in breeding qualitative traits as the genetic ratios are predictable making them easy to select. Many disease resistance traits are qualitatively inherited. Breeding methods such as backcross method are very effective for improving qualitative traits such as disease resistance. The challenge arises when dealing with quantitative traits as many genes are involved and the environment plays a role in the expression of these traits. The best example of a quantitative trait would be yield, where many (10-100) genes are involved, each having a small effect and the effect is not absolute in that environmental factors influence expression. Most important characteristics in crop plants are quantitatively inherited. Breeders use the term 'heritability' to express that portion of a quantitative trait that is under genetic control. A qualitative trait (flower color) would be 100% heritable whereas quantitative traits have significantly lower heritability values (estimates). For example, yield usually falls in the 10-15% heritable range, plant height ranges from 35-40%, seed size ranges from 65-75% heritability. So the gain from selection will always be lower for yield than for seed size as the heritability (genetic portion that breeder selects) is lower. Therefore progress for yield is rarely dramatic, but a low steady constant gain. Quality traits have variable heritability depending on the trait being measured but usually they range from 35-45% whereas seed color is highly heritable and easy to select for and fix in new varieties. In order to improve quantitative traits, breeders must work in the field as the environment affects trait expression, trials must be repeated over years and locations and all field trials are replicated in statistical designs to help reduce the effect of extraneous environmental factors that are outside the control of the breeder. Factors such as variation in soil type, disease, insect, or weed pressure, fertility differences in soil profiles, crop rotations etc all influence a trait such as yield making it difficult for breeders to truly identify the genetic from the environmental portion as only progress can be made by identifying and selecting the genetic portion. In addition to the slower progress in improving quantitative traits, progress is less predictable and even elusive as some traits may not combine well following crossing due to the complexity of genetic interactions affecting the trait.

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Additional information on the Principles of Mendelian genetics, and Meiosis and Plant Breeding Methods are illustrated in web sites below.

<http://www.dnalc.org/resources/animations/index.html>

<http://www.hrt.msu.edu/pbgp/Links/plantbreedingintro.html>

<http://www.youtube.com/watch?v=iCL6d0OwKt8&feature=related>

<http://cuke.hort.ncsu.edu/gpb/>

<http://theagricos.com/plant-breeding/>

<http://www.ndsu.edu/pubweb/~mcclean/plsc431/mendel/mendell.htm>

<http://vcell.ndsu.edu/animations/>

Description of breeding program for a new bean cultivar

The overall goal of a breeding program is to improve specific characteristics of a bean variety without compromising other characteristics that the variety possesses. Successful breeders must strike a balance by enhancing traits of economic importance while retaining other traits at an optimum level as these also contribute to the overall value of the variety. The choice of traits to improve is based on perceived needs of producers, consumers and what is achievable based on the biology and genetics of the crop. Breeders establish a number of attainable short and long term objectives that meet the approval of the industry and are supported by colleagues in related disciplines.

The breeding process to develop a new bean variety is fairly standard, similarly structured in most breeding programs and takes 10-years from time of the initial cross to the sale of certified seed of the new variety to commercial bean growers. The process is divided into three 3-year steps that are described as the early generation selection step; the yield and quality evaluation step; followed by 3-years state and federal produce certified (blue breeding programs have of development, from or lines in preliminary to this situation as having new varieties will be The 10-year lag only initiated or if a new trait was being introduced. Predicting the actual appearance of a new variety is not a science as the interaction of genetics and the environment (GxE) plays an unpredictable role as a fault may appear in an otherwise strong candidate.

Year	Activity
1- 3	Crossing & Early Generation Single Plant Selection - F2-F5 generations
4-6	Yield Testing in Replicated Plots – Canning
7-9	Seed increases, Breeder, Foundation, Certified – continue yield testing and canning
10	Certified Seed Available to commercial growers

of seed multiplication under approved guidelines to tag) seed for sale. All active materials at different stages elite lines to early-generation yield testing. Breeders refer the ‘pipeline’ full, so that emerging every few years. applies if a program is being

Beans are a self-pollinated crop in contrast to a cross-pollinated crop like corn (corn is wind-pollinated; alfalfa is insect-pollinated) which biologically dictates the type of variety that can be produced for sale to farmers. In the case of corn, hybrids, which are the product of a cross between two diverse parents, are sold to farmers. In the case of self-pollinated crops like bean, soybean, and wheat only pure line varieties are sold to farmers. Pure line varieties are developed from a cross and taken through 10-generations of self pollination to ensure that they are pure breeding. Pure line varieties take longer to develop than hybrids. In some self-pollinated crops like tomato, hybrids are sold as the value of hybrid seed is great and tomatoes produce large

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quantity of seed (100+seeds) from a single cross whereas a single cross only produces 4-6 hybrid seed. Beans are cleistogamous which means that they are already self-pollinated when the flower bud opens. As a result it makes the task of producing hybrids difficult and economically impractical to produce sufficient quantities of hybrid seed for sale.

Initial Crossing, Early Generation Selection; Years 1-3:

Year 1: Breeders make many different crosses between diverse parents to produce the first filial (F1) generation in the greenhouse. Crosses are made to complement attributes of the two parents: cross a commercial variety lacking disease resistance with an unadapted bean line that has a gene for resistance. Crosses may involve 3 or 4 parents as a means to introduce more genetic diversity. The decision on the choice of parents may come from other colleagues, farmers, industry reps or from staff in the breeding program. Immature flower buds (Fig 6) are opened using tweezers and pollen from the male parent is transferred to the stigma of the female parent and the bud is tagged. Immature anthers on the female parent are usually not removed as the bud is tripped to separate the female stigma from the male anthers. A descriptive guide to bean crossing is available at http://www.css.msu.edu/bean/PDF/bean_pollination.pdf. All crosses



Fig 6. Open bean flower is already self-pollinated. The immature bud in the background is used to make a cross pollination

are made in the greenhouse, usually in the fall and the F1 generation progeny is allowed to self-pollinate to produce the next generation – F2 seed in the greenhouse the following spring. At this point the breeder can follow any number of different breeding methods to advance and select new lines, but the most common is the Pedigree breeding method. Other methods such as backcross breeding or recurrent selection are used to meet very specific objectives and the new marker-assisted selection (MAS) system can also be deployed. Breeders may combine breeding methods to save time and/or resources.

Year 2: The F2 seed is space planted in the field and the different crosses are kept separate. Selection is initiated in the F2 generation and the single plant selections are harvested separately. Selection is based on the best combination of agronomic and seed characteristics in individual plants and freedom from endemic diseases. In order to speed up the process the next F3 generation is planted as a plant-row (single row) in a winter nursery in Puerto Rico. Selection is practiced on a row basis for similar characteristics and the best plants in the row are combined.

Year 3: The F4 seed (next generation) is planted in field in MI and selected on a plant-row basis. Individual F4 plants are selected and harvested separately and sent to PR as F5-generation plant rows. Selected rows are harvested and returned to MI where they will be planted in preliminary yield trial (PYT) plots as F6 generation lines. During the grow-outs in PR, remnant seed of the same lines are tested for reaction to different diseases in the greenhouse in MI and in some cases MAS is practiced on the same lines. Only those lines with adequate levels of disease resistance are selected in PR. This concludes the first trimester of the program.

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Yield Trials and Canning Quality Evaluations Years 4-6:

Years 4-6: The F6 generation lines selected from plant row in PR are planted in replicated yield trial plots in a randomized statistical design (lattice) in the field in MI. Plots are usually 4-rows with 3-replicates (cover photo). Data is collected on a range of agronomic traits; flowering maturity, lodging, height differences between plots symptoms on the cover harvested, seed yield, seed are collected, tabulated, top yielding 50% of chosen for canning. Those satisfactorily are advanced

<u>Year</u>	<u>Generation</u>	<u>Activity</u>	<u>Location</u>
1- Fall	Parents	Crossing	GH
1- Spring	F1 plants	Selfing	GH
1- Summer	F2 plants	Selection	Field - MI
2-Fall &Sp	F3 rows	Selection	Field - PR
2-Summer	F4 rows	Selection	Field - MI
3- Fall &Sp	F5 rows	Selection	Field - PR
3-Summer	F6 yield trial	Selection	Field - MI
4- Summer	F7 yield trials	Selection	Field - MI
5- Summer	F8 yield trials	Selection	Field - MI
6- Fall	Possible Release of Variety		

and any incidence of [observe maturity and some disease photo]. Plots are machine size, and moisture data and analyzed. Only the entries in PYT are lines that can for testing in advanced

yield trial (AYT) plots in year 5. A few of the best candidates may be tested at more than one location. AYT consist of similar plot arrangements but with 4-replicates. Similar agronomic data is collected as in year 4 and the 50% top-yielding lines are canned. Those lines that continue to can satisfactorily are tested a third year in AYT at multiple locations. One of the locations is managed to promote white mold development in order that the lines can be assessed for their reaction to this serious disease. Agronomic and canning data is collected in year 6 and a decision is made to continue testing and advancing the best lines in future years at more locations. Testing may be extended to other programs outside MI and if a line looks like a strong candidate for variety release a sample of greenhouse seed of that line is sent out west to WA or ID to initiate a seed increase as pre-breeder seed.

Seed Certification; Years 7-9:

Year 7: When a decision is made to release a new variety the next step is to multiply seed from limited quantity (few pounds) to sufficient quantity for marketing to commercial growers. The seed law in MI designates a 3-class system of Breeder, Foundation, and Certified Seed – in that order. Certification of bean seed of MSU varieties follows the 3-class system even though other states recognize a 4-class system that includes a Registered class. The breeder seed is the property of MSU and is maintained by the breeder or a designated representative. Since bean seed is produced in the western US to reduce/eliminate problems with major seed-borne diseases such as anthracnose and common bacterial blight, breeder seed is produced under contract in the west since MSU does not maintain university farms outside of MI. Federal and state laws for field, and lab inspections ensure that the seed is genetically pure, free from diseases, pests, and other crops or weeds.

Year 8: Foundation seed is produced from Breeder seed under contract with Michigan Crop Improvement Association (MCIA). Seed quantities depend on anticipated demand for the new variety. The majority of Foundation seed is produced in WA or ID, but limited production is done in MI, as the risk of infection from disease is higher. Foundation seed fields are inspection by representatives of the Dept of Agriculture in each state. Fields can be rejected if they lack purity, do not conform to the variety description, or are contaminated with other crops, weeds, a/o other off-type varieties. Lab tests are performed on the seed to ensure purity, germination levels and freedom from pests and diseases. The breeder may designate a portion of a foundation

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seed field as breeder seed for production next year. All foundations seed fields are inspected by the breeder (or representative) and a member of MCIA staff.

Year 9: Certified seed is produced from Foundation seed following the same guidelines as was detailed for Foundation seed. Seed that meets certification standards is tagged (blue tag) with name of variety, % germ, freedom from crops, weeds, pests and name of the producer. The quantity of seed produced is based on anticipated demand for that seed and the general availability of foundation seed. Seed is produced under state and federal guidelines in a number of states where demand for the seed is expected.

Year 10: Certified seed of new variety is sold to commercial bean producers to produce tablestock beans for processing and sale overseas. During years 7-9, when seed is being multiplied, breeders and agronomists continue to yield test and evaluate the canning quality of the new line/variety in a number of states through Cooperative Dry Bean Nurseries. This provides exposure for the new line/variety to multiple environments and also determines where it is best adapted and where it should be marketed.

Release procedure for a new variety: The procedure to release a new variety generally takes place after year 6. At that point in its development the generally recognized potential of a new breeding line is recognized and its potential or need in the marketplace is anticipated. At MSU all new bean varieties are evaluated by at least two committees consisting of other breeders, agronomists, industry, extension staff and advisors from the university intellectual property office. The final decision to release a variety rests with the Director of the Michigan Agriculture Experiment Station. When approved for release the office of MSU Technologies seeks potential licensees from among those who would be interested in growing and marketing the variety and providing a royalty back to the university. Every effort is made to ensure that the variety receives the optimum exposure and the licensee may be a single company or a broader group of interested parties. Small market licensed and marketed by a single company may be commercial class such as maintains all rights to the responsibility to produce production of foundation the responsibility of the through MCIA for seed University usually protects all new varieties through Plant Variety Protection (PVP) Act, where the uniqueness of the variety must be provided and accepted. The variety is protected from illegal propagation for 20 years under PVP.

The Plant Variety Protection Office (PVPO) administers the Plant Variety Protection Act (PVPA), by issuing Certificates of Protection in a timely manner. The Act provides legal intellectual property rights protection to breeders of new varieties of plants which are sexually reproduced (by seed) or tuber-propagated.

specialty beans are best a single company whereas too restrictive for a large black or navy bean. MSU variety, along with breeder seed but the and certified seed will be licensee who may work production needs. The

A list of all protected dry bean varieties are available at the PVP web site listed below.

<http://apps.ams.usda.gov/PVPO/CertificateDatabase/cropsearch2.asp?pagenum=1&Crop=Bean,field>

<http://www.ams.usda.gov/AMSV1.0/ams.fetchTemplateData.do?template=TemplateC&navID=vpomainpage&rightNav1=vpomainpage&topNav=&leftNav=&page=PlantVarietyProtectionOffice&resultType=&acct=plntvarprtctn>

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CLASSICAL APPROACHES, TECHNIQUES AND OUTCOMES USED IN BEAN BREEDING

Priority setting and techniques used in bean breeding are illustrated in the text and fig 7 below. Approaches include traditional genetic and breeding methods combined with new molecular marker technologies to meet the needs of producers and consumers alike. Outcomes in terms of yield gains, architectural improvements and consistent quality of new varieties are illustrated.

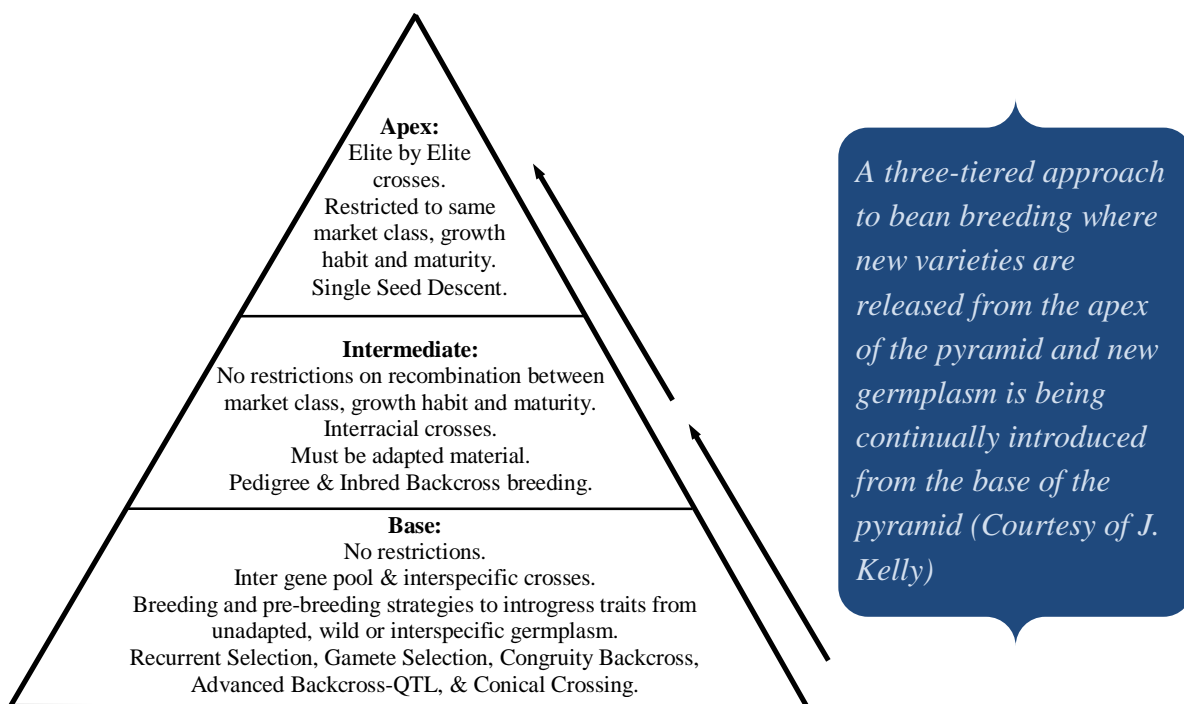


Fig 7. BEAN BREEDING PYRAMID

PRIORITY SETTING IN THE DRY BEAN BREEDING AND GENETICS PROGRAM AT MSU

- **Breeding for yield, adaptation, plant architecture, stress tolerance and sustainability of production in navy, black, pinto, great northern, light red kidney, dark red kidney, cranberry, white kidney, yellow eye, soldier, small red and pink bean market classes.** Different classes present unique problems in improving yield and plant architectural traits. For example, it has proven impractical to develop early maturity in upright indeterminate (Type II) growth habit because the plants pod lower to the ground in the navy bean class. A negative linkage between desired medium seed size and type II growth habit has slowed progress towards combining both traits in pinto and great northern beans. Since kidney and cranberry beans belong to the Andean gene pool distinct from Middle American gene pool (includes navy and black beans), the effective utilization of genetic advances made in navy germplasm as a genetic donor for yield improvement in kidney and cranberry beans has been hindered.

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DISEASE RESISTANCE BREEDING

- Genetics and breeding for disease resistance in the same ten commercial classes. The occurrence of new races of common pathogens and the lack of adequate levels of resistance in non-traditional classes of beans require a major breeding effort. The major pathogens endemic in the humid Midwest include rust, anthracnose, mosaic virus, white mold, root rot and bacterial blight. Genetic resistance to these pathogens is being evaluated and when available will be incorporated into different seed classes. Since new races of anthracnose, rust and mosaic have been identified, the pyramiding of distinct sources of resistance factors in single genotypes is being pursued. To facilitate the disease resistance breeding, molecular markers tightly linked to the different resistance genes are being developed to permit gene pyramiding and allow for simultaneous selection of multiple disease resistance.

CANNING QUALITY BREEDING

- Selection for dry seed and processing quality traits. Since beans are consumed directly by humans, they must meet minimum standards for both dry seed and processed seed quality. Over 90% of navy and kidney beans are canned and an increasing large percentage of pinto and great northern beans are now processed. In addition to selection for dry seed traits such as size, shape, and color, all new germplasm must also be evaluated for processing characteristics. Characteristics include: hydration ratio, washed drained weight ratio, processed color, and shear press texture. The complex inheritance of these traits coupled with the delay due to inbreeding prior to evaluation suggests the possibility of developing linked markers to assist in the early generation selection for improved processing quality.

MOLECULAR MARKER TOOLS

- Development of molecular markers to facilitate the selection of traits important in the breeding program. Molecular markers linked to nine major resistance genes which control three pathogens, have been developed. The breeding program is re-orienting its focus to better use these markers in routine disease resistance breeding. Research is continuing to develop markers associated with more complex quantitative trait loci (QTL) controlling drought resistance, canning quality, resistance to root rots and white mold. Marker-assisted selection (MAS) studies have proved useful in the selection of bean genotypes with enhanced resistance to white mold.

INTERNATIONAL ACTIVITIES

- International research funded through the PULSE Collaborative Research Support Program (CRSP) has focused on the use of MAS in breeding beans for drought and disease resistance in Mexico, and Guatemala and research continues in Rwanda and Ecuador. The work will continue and be expanded to provide access to wild bean germplasm that may carry potentially useful traits to enhance future bean breeding efforts.

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Public Bean Breeding Programs in the U.S.

The first bean breeding program in the U.S. was initiated at MSU in the early 1900's. Highlights and history of that program (Table 1), variety releases (Table 2) over the decades can be found on line at <http://www.css.msu.edu/bean/>

The most recent program was initiated at NDSU in the early 1980's.

<http://www.ag.ndsu.nodak.edu/plantsci/breeding/drybean/>

Other public breeding programs are location at University of Nebraska, Scottsbluff

<http://www.nebraskadrybean.com/research20.htm>

Colorado State University, Fort Collins,

http://www.colostate.edu/programs/wcrc/pubs/research_outreach/fndbeans.htm

and the University of Idaho, Kimberly established in 1925,

<http://www.kimberly.uidaho.edu/beans/>

Four federal programs focus on different aspects of bean improvement. USDA-ARS programs at Prosser, WA, Beltsville MD, Mayaguez, PR and East Lansing, MI work on different research aspects at these locations – germplasm enhancement at Prosser, Beltsville and Mayaguez; disease, rust screening at Beltsville, Genetics Quality traits at East Lansing, and Genomics and Germplasm Conversion at Mayaguez. These programs contribute enhanced germplasm to both public and private breeding programs.

<http://www.ars.usda.gov/pandp/people/people.htm?personid=3848>

<http://www.ars.usda.gov/Research/docs.htm?docid=4337>

<http://www.ars.usda.gov/is/AR/archive/jun06/beans0606.htm>

<http://www.ars.usda.gov/pandp/people/people.htm?personid=40697>

USDA-ARS bean research activities recently summarized;

<http://www.ars.usda.gov/is/AR/archive/may10/May-June2010.pdf>

Unique characteristics and limitations or constraints for beans

Beans are a non-GMO crop. Beans will remain a non-GM crop until consumers worldwide accept GM-bean products and scientists can successfully genetically engineer (transform) bean plants to express a foreign gene(s). As a result bean breeders can only improve future bean varieties for traits that exist within current bean germplasm (includes cultivated, landrace and heirloom beans, related species and wild species). Traits such as herbicide resistance cannot be introduced from other species of plants, microorganisms or from distantly related species (soybean) as this would require genetic engineering. Genetic characteristics can only be transferred through manual cross-pollination which restricts the genetic variation does not gain that can be made. If genetic germplasm exist for a trait in the array of bean improve the trait. available, bean breeders cannot new genetic One exception might be to create genetic variation through mutation breeding, but any useful outcome of this methodology is highly unpredictable. Despite these limitations, yield gains (Fig. 8), enhanced levels of resistance to many major pathogens, more upright plant architecture, and modest canning quality improvements have been made by breeders in a range of commercial bean seed types.

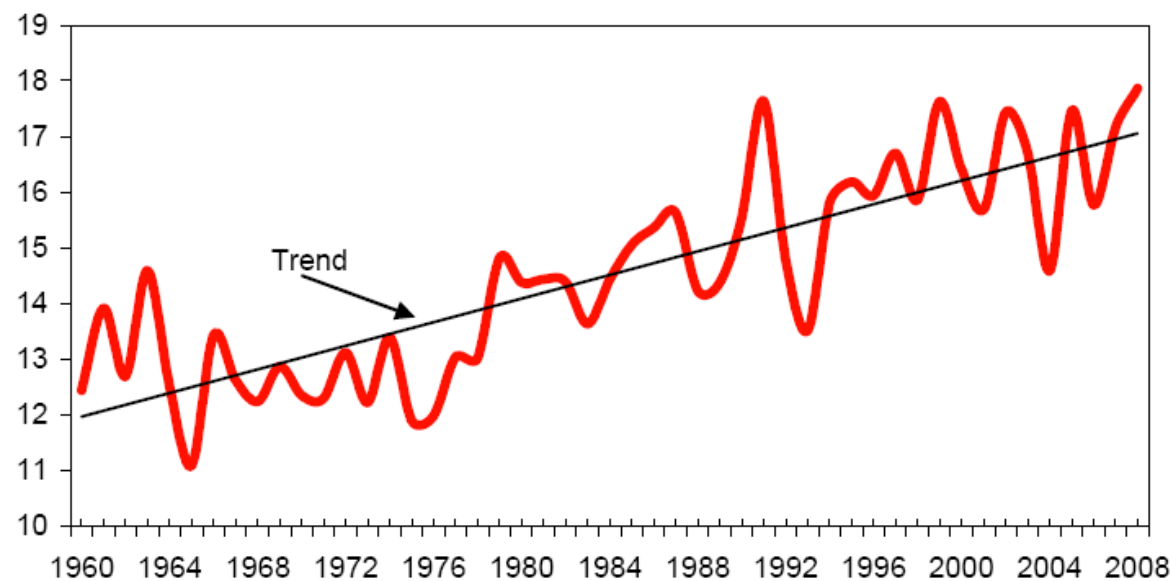
“The Sanilac navy bean variety released in 1957 was developed through X-ray mutation breeding”

The Story of Bean Breeding

Figure 10

U.S. dry beans, all: Average yield per acre, 1960-2008 1/

Cwt/acre 1/



1/ Cwt = 100 pound units.

Source: USDA, National Agricultural Statistics Service, *Crop Production*.

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Economic Research Service, USDA

Fig 8. Yield gains are attributed to a combination of genetic improvement and management. In most crops genetic gain contributes 50% of the total gain in productivity.

Traits vary in importance depending on whether you are a producer, dealer or processor. All have different needs and expectations of bean varieties. Producers are interested in yield, ease of harvest, standability (lodging resistant), freedom from diseases, whereas the elevator industry are interested in seed quality traits, seed free of splits, insect damage, uniform color, shape, size, and moisture content, and the processing industry is interested in quality, processor yield, hydration ratios, color, freedom from splits, drained weights, and visual appearance. Combining all of these diverse traits into a single cultivar is not easy as breeders cannot focus on a single trait but must deal with an array of traits at the same time. Some traits are negatively correlated. For example, early maturity and high yields are not easy to combine. Drought tolerance may require deeper roots that take energy away from foliar growth and ultimately yield.

One area where substantial progress has been made over the last decades has been in the development of upright bean varieties, similar in plant architecture and structure to soybean. These erect upright types are resistant to lodging, pod higher in the plant canopy and allow growers to direct harvest the crop with minimum seed loss, not possible with traditional short bush or prostrate vine type varieties (Fig 9). Direct harvest represents considerable saving in time, personnel and equipment for the grower.

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Fig 9. Three growth habits of beans grown in the U.S. Determinate bush (left) is typified by kidney beans; Upright short vine suitable for direct harvest (center) typified by black and navy beans; viney prostrate indeterminate type (right) is typified by traditional pinto and great northern beans grown in the west. J. Kelly

Standability varies by seed class with the most erect types available in navy and black bean classes. Improved architectural types exist in pinto, great northern, small red and pink beans and growers need to select the variety that best suits their production needs in these classes. The plant habit currently available in the large seeded kidney and cranberry classes is a determinate bush type that is not suited for direct harvest. Breeders have not been successful in changing the plant habit in these classes and major efforts to do so have not been made as direct harvest may result in more seed checks that would make these classes unmarketable. Developing an upright vine habit in kidney beans would also result in a greater range of seed sizes on the same plant which would be equally undesirable. In the bush habit the plant aborts seed in upper pods in an effort to retain more uniform larger seed due to competition for nutrients. The plant may produce fewer beans but size is larger and more uniform. Similar compensation in smaller seed classes is not as obvious. No discussion of important economic traits would be complete without a discussion of disease resistance. Given the wide range of pathogens that attack beans, breeders focus on diseases of major importance in their production area. In the east and Midwest, foliar bacterial and fungal pathogens are the most serious, whereas, root pathogen and insect vectored viral pathogens are more problematic in the western U.S. Progress is made on a local level but some disease such as white mold have proven very difficult to control as resistance is quantitative and no high levels of resistance exist in bean germplasm collections. A GM approach may offer the best potential to control this disease <http://www.whitemoldresearch.com/HTML/drybeans.cfm>.

With the change and interest in direct harvest, breeders now include in the breeding/selection process, a direct harvest step to assure that all new varieties can be successfully harvested without major problems of seed loss, due to low pod placement, lodging due to weak stem strength, seed splitting due to actual threshing as less plant mass (no roots) goes through the combine. Yield is still used as the major selection criteria to advance new breeding lines in the program but direct harvest provides the opportunity to eliminate those lines that for whatever reason fail to meet expectations in ease of harvest, with minimum harvest loss, or lower yield as a result of direct harvest and/or increased incidence of seed splitting due to the actual direct harvest process.

The Story of Bean Breeding

Germplasm access

Beans are largely bred at public institutions, so there is relatively free exchange of germplasm between programs. This continues today with the addition of a paper trail – MTAs for material transfer agreements are now required by many public institutions. Breeders freely share germplasm developed in their programs for use as parents in both public and private breeding programs. A list of bean varieties released by public institutions is shown on Table 2. In addition bean varieties released and protected by PVP Act are listed on line and can be used as parents in a crossing program.

[<http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3002796>]

If used as a recurrent parent in multiple crosses (more than 2-crosses), permission should be requested from the owner as that progeny would be regarded as ‘essentially-derived’ and would bear too much resemblance to the original parent. Likewise GM beans would fall into the essentially-derived class and require permission or licensing from the original owner of the variety. Germplasm collection of 11,000 accessions is maintained by the USDA-ARS, Pullman WA and a seed sample for research or breeding is available on request. Data collected on accessions is returned to the curator of the collection and is available on line on the Germplasm Resource Information Network (GRIN) System [<http://www.ars-grin.gov/>]. A larger bean germplasm collection of 25,000 accessions is maintained at International Center for Tropical Agriculture (CIAT), Cali, Colombia

http://www.ciat.cgiar.org/AboutUs/Documents/synthesis_bean_program.pdf.

Current policy on obtaining seed from CIAT can be found on the BIC web site [<http://www.css.msu.edu/bic>]. CIAT maintains active breeding programs in Central America and East Africa and activities are all donor supported. Most recent activities (support from Gates Foundation) have been on the biofortification of beans for enhanced levels of Zn and Fe.

Private sector bean breeding programs include ADM Edible Bean Specialties and Seminis that have a major investment in bean breeding, and a few smaller companies that focus exclusively on bean breeding (Gentec, Provita). The latter contract research with seed companies and/or elevators interested in vertical integration – exclusive sale of bean seed, purchase of tablestock beans from same growers for marketing in the fall. A positive collaborative working relationship exists between public and private sector breeding programs.

Current recommended varieties, in different commercial classes, release dates, agronomic and production information, electronic extension bulletins are provided on individual web sites from different state breeding programs listed below.

<http://www.css.msu.edu/bean/>

<http://www.maes.msu.edu/ressta/saginawvalley/bean1.html>

http://www.css.msu.edu/VarietyTrials/DryBean_HomePage.html

<http://cuke.hort.ncsu.edu/cucurbit/wehner/vegcult/beandry.html>

<http://www.ag.ndsu.nodak.edu/plantsci/breeding/drybean/>

<http://www.nebraskadrybean.com/research20.htm>

http://www.colostate.edu/programs/wcrc/pubs/research_outreach/fndbeans.htm

<http://www.kimberly.uidaho.edu/beans/>

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REVIEW OF MOLECULAR TOOLS AND APPROACHES IN BEAN BREEDING

The new era of molecular genetics has impacted all plant breeding programs. With the advent of recombinant DNA technologies, new tools and avenues of research were opened up for plant breeders. These tools include access to molecular markers and the science of genomics which is largely based on the ability to sequence the DNA of crop genomes to determine the gene order and genetic basis of the crop. The DNA of all crops is organized in linear structures known as chromosomes (Fig 10). Chromosomes of most crops are visible under a light microscope and can be seen to pair during meiosis. The number of chromosomes varies between crops and is based on the number of homologous chromosomes that pair during meiosis. In beans there are 11 pairs of chromosomes compared with 20 pairs in soybean. Since the DNA is arranged in a linear fashion, the genes along the chromosome are either adjacent to or distal from other genes. When two genes are adjacent on a chromosome they are linked. If the genes are on different chromosomes they are not linked and assort randomly during meiosis. Genes are not uniformly distributed along the chromosome but most active genes (those expressed) are clustered toward the extremities of the chromosome arms. If two genes are tightly linked (close together), it may be impossible to break that linkage, so traits controlled by these genes are always associated together. This may be useful if traits are valuable but negative linkages impede breeding progress.

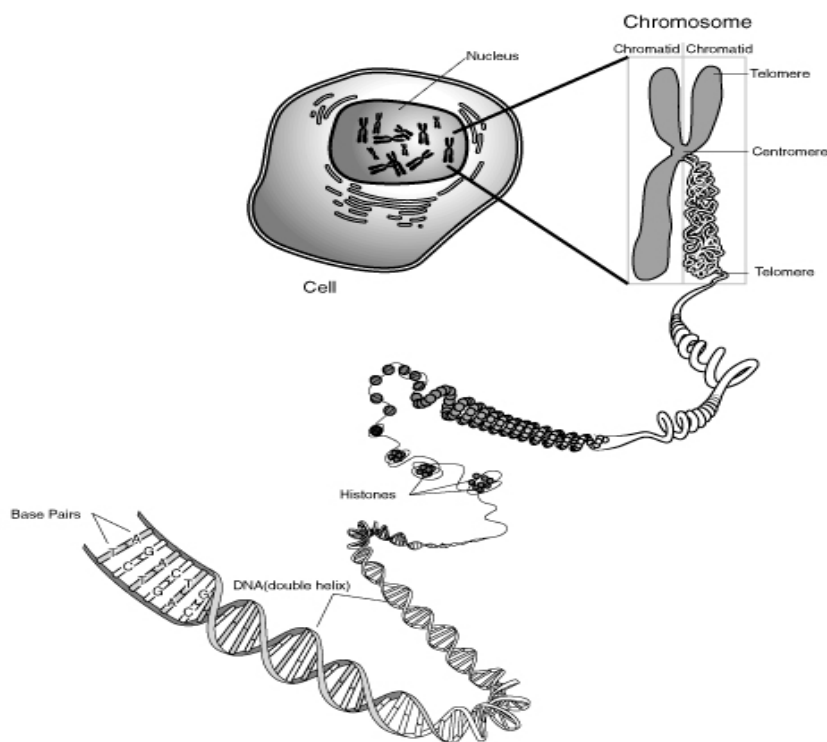


Fig 10. Chromosomes, structure, arrangement and DNA double helix. Beans have an estimated 588 million base pairs. Courtesy of the National Human Genome Research Institute by Darryl Leja

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Geneticists have been able to use the physical attribute of linkage to help study gene order and function. Regions of the DNA that can be identified are known as markers as they can be visualized following amplification (using polymerase chain reaction-PCR) and separated using gel electrophoresis. Since DNA is negatively charged it migrates to the positive electrode when an electric field is applied, so DNA fragments can be separated based on charge and size. DNA fragments appear as bands on the gel under UV light. Markers of known size (base pairs) can be identified and such markers are used in forensic science to characterize human DNA. Beans can be similarly characterized with molecular marker or tags and these can be mapped to regions of the bean chromosome. When a marker is near to a gene of interest it is linked to that gene so breeders can follow that gene in a segregating population based on the presence or absence of the marker. As a result, the use of marker-assisted selection (MAS) has been developed as a plant breeding tool to allow breeders to indirectly select for a trait of interest by simply selecting for the DNA marker.

molecular markers exist properties that make it depending on the crop knowledge available for closely linked a gene is to useful the marker. All some distance from the molecular genetics DNA sequence

- RFLP – anchor points for mapping - radioactivity
- RAPD – cheap, convenient, use PCR protocol
- SCAR - wider utility across labs and maps
- AFLP – highly polymorphic, useful in diversity studies
- STS – Sequence Tagged Sites = SCAR from AFLP
- SSR – Simple Sequence Repeats, Microsatellites
- SNP – Single Nucleotide Polymorphisms - sequence
- CAPS – Cleaved Amplified Polymorphic Sequences
- TRAP - Target Region Amplified Polymorphism
- SRAP - Sequence Related Amplified Polymorphism

Different types of and each has unique more or less useful and the genetic that crop. The more a marker, the more markers are linked at gene. As the science of advances and more information is available,

geneticists are able to identify specific genes and their sequences. The ideal marker system is one where the marker is part of the actual gene. This type of marker is known as a single nucleotide polymorphism (SNP) and the difference is based on nucleotide change that can be detected which changes the function of the gene. For example a single nucleotide change exists between a rice variety that shatters and one that does not. Currently all private corn and soybean breeding programs have developed SNP marker platforms to assist breeders in identifying traits of economic importance and providing a tool to more quickly combine useful genes in new varieties. All of these technologies are tools to assist the breeder to conduct MAS and do not involve genetic engineering which is the topic of the next section.

Having the ability to indirectly select for a trait without actually evaluating the trait has benefits for the plant breeder. For example breeders could select for disease resistance trait without actually working with the disease, assuming that a tightly linked marker is available. Disease quarantine may restrict working with exotic diseases (soybean rust) at certain locations. Breeders can more easily combine multiple genes conditioning resistance using MAS as the phenotype remains constant. The area where MAS will ultimately have the most impact is working with quantitative traits. Having access to molecular markers scattered over the crop genome and mapped on different chromosomes, breeders have a method to map the location of all the genes that constitute a quantitative trait. The terminology for this process is known as QTL mapping. QTLs are quantitative trait loci and these are regions on the chromosome where a gene resides that is partially responsible for controlling that quantitative trait. QTL is a statistical term as the location (loci) is based on statistical probability that a portion of a quantitative trait resides at that location. The statistical parameter is known as a LOD score and scores of 3 or

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greater indicate the presence of a QTL. In addition to location (loci) the size and effect of each QTL is known as each is different. Some QTLs will have a major effect on a trait and may control over 50% of the genetic variation of the trait. Moderate QTL effects would range from 25-45% and minor QTL would control only 10% of the variation for a trait. Knowing the location (marker location) of major QTL controlling a complex quantitative trait provides breeders with a method to combine major QTL into new varieties in order to enhance a quantitative trait. Minor QTLs may be ignored in this process. In selecting for yield breeders may be able to combine 2 or more QTLs for yield (computer driven) and reduce the number of actual field trials. In all indirect selection studies that involve some form of MAS, it is critical that breeders verify that the newly selected material fully expresses the trait of interest as linkages are not absolute and can be broken due to crossing-over in a normal breeding/crossing program. QTLs have become the new 'genes' in plant breeding vernacular, as many important traits are quantitatively inherited.

Since the DNA of all crops is arranged in linear chromosomes, breeders/ geneticist have been able to develop genetic linkage maps for most crops based on the order of markers on these chromosomes. The chromosomes are physical structures that are not easily visualized and the chromosomes of bean are so small they are indistinguishable. Since gene order is based on linkage geneticists refer to that gene order as linkage groups which coincide with the actual number of chromosomes. In bean there are 11 linkage groups. Knowing the location of a gene or QTL on a linkage group is very important to breeders as it provides insights into the other genes that are present on the linkage group and may be linked. A map of the linkage groups of beans and the genes/QTL that are mapped to individual linkage groups is posted on the BIC web page. Correspondence between linkage groups and chromosomes is also shown on this site. [<http://www.css.msu.edu/bic/Genetics.cfm>]. Given the common origins of beans and soybean, genetic maps allow breeders to compare gene order for traits between different species. Despite the separation in time (18 mya), there is considerable synteny between gene order of bean and soybean (Fig 11). The implication of this information is that research on traits in soybean could be applied to bean. For example more research has been conducted on soybean than bean and more information exists on resistance to white mold. If QTLs are identified for resistance in soybean, bean breeders may be able to use that information to 'fish' for similar resistance QTL in bean germplasm. Since low oligosaccharide soybean lines exist, breeders are using those markers to see if they can find similar low oligosaccharide types in bean.

The area of translational genomics applied to beans is expected to expand through the BeanCAP project. The goal of the Federal Applied Plant Genomics Coordinated Agricultural Project (CAP) is to focus on large-scale application and translation of genome discoveries and technology for U.S. crop improvement. The specific goal of the BeanCAP will provide new tools and research directions for all market classes of this important nutritional and commodity crop. These tools will also have a broad impact on improvement for all agronomic traits. The first market-class-specific markers will be a major outcome affecting all bean research. When genotypic data, generated by using these markers, is coupled with nutritional profiling data, also generated by the project, species-wide and market-class-specific loci affecting the nutritional traits will be discovered. This will set the stage for nutritional improvement of common bean for future years. All public US bean breeding programs will also be supported by 1) a genotyping program that will aid the discovery of genetic factors controlling traits of local agronomic importance; and 2)

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conversion of high throughput markers into low cost makers for day-to-day use in breeding programs. The nutrition, genetic, and genomic scientists will coordinate multiple social networking communities that will utilize high-quality animations and other multimedia to educate the general public and educational communities about the biology of nutrition and how genetics/genomics technology assists with the improvement of nutritional traits. The BeanCAP will also initiate a modern plant breeding training program focusing on early career recruitment and practical breeding/genomics training that illustrates, as an example, how the integration of genomic and phenotypic data can be used to improve nutritional traits in plants. These programs will be advertised at multiple agricultural education conferences. The goal is to provide a stream of students interested in filling the plant breeding human resource pool. The BeanCAP is comprised of a national team of 26 researchers from 16 institutions including all of the public common bean breeders, plant pathologists, and extension personnel in the U.S.

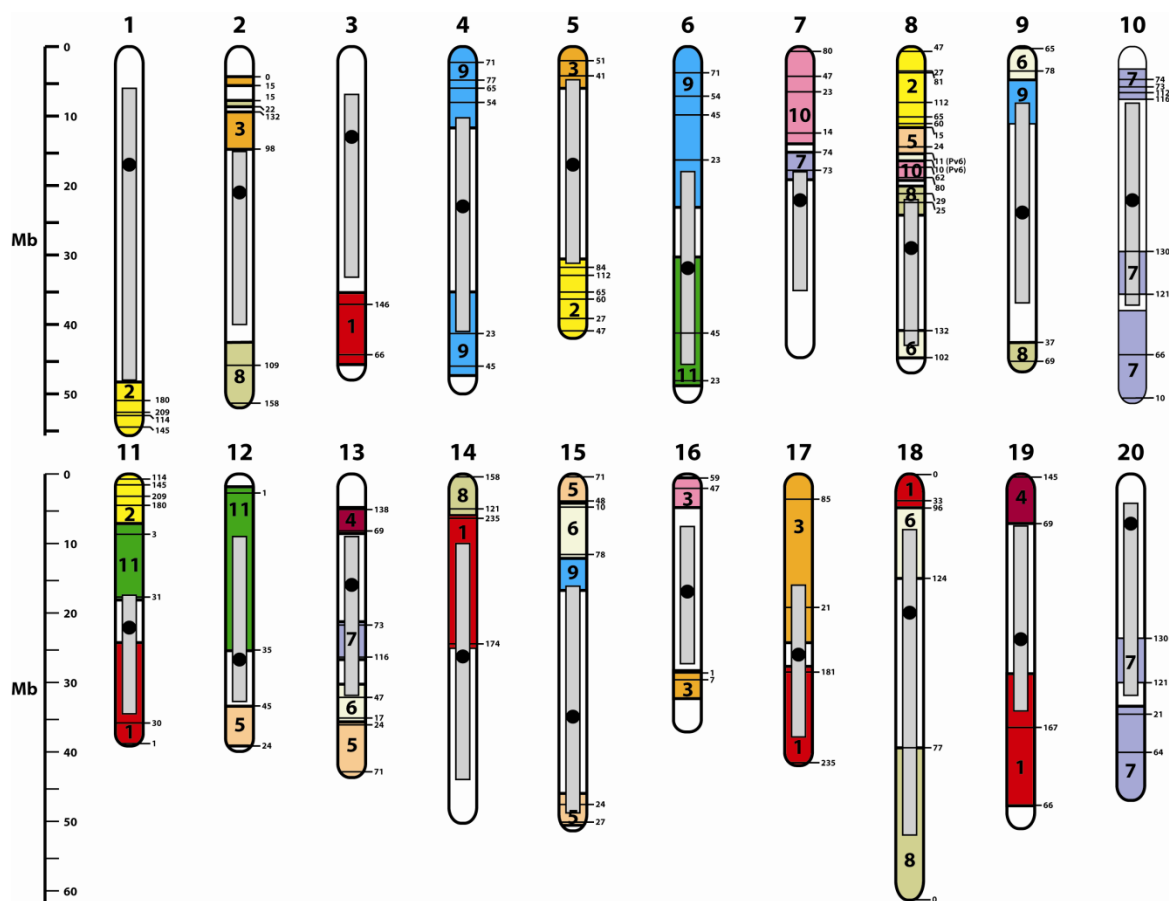


Fig 11. Twenty chromosomes of soybean (numbered 1-20) and the scale (left) refers to length of each chromosome based on number of bases in millions (Mb). Colored regions represent syntenic (similar gene order) regions with portions of the 11 chromosomes of bean, numbered 1-11 within each soybean chromosome. For example soybean chromosome no 1 is 55 Mb in length and a small portion from 48-55 Mb has sequences similar to chromosome no 2 of beans. Note the duplication of bean chromosomes on the different soybean chromosome- confirming that soybean genome duplicated following separation from a common bean ancestor over 18 mya; Figure courtesy of P. McClean – NDSU

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CURRENT DEVELOPMENTS AND ISSUES OF GENETICALLY MODIFIED ORGANISMS (GMO)

Genetic modification (GM) or genetic engineering involves the transfer of genes from another organism or species into a crop plant genome, and then regenerating a whole plant from the transformed tissue or cell. Currently, one of the most widely used method for transferring genes into plants is *Agrobacterium*-mediated plant transformation. *Agrobacterium* is a naturally occurring pathogenic bacterium in the soil that has the ability to transfer its DNA into a plant's genome. *Agrobacterium* serves as a vector that is capable of carrying desired genes into the plant. The engineered genes are inserted into the *Agrobacterium* vector and enter the plant by the bacteria's own internal transfer mechanisms. Transformation is typically done on a small excised portion of a plant known as an explant. This small piece of transformed plant tissue is then regenerated into a mature plant through tissue culture techniques. Success with bean transformation has been very limited. The few successful GM or transgenic bean varieties have been developed by physical methods that circumvent problems with plant regeneration. In Brazil, researchers used particle bombardment protocol to insert herbicide (*bar* gene) and virus resistance (RNAi) into the pinto bean variety, Olathe. This work was recently reported as 'First transgenic Gemini virus resistant plant in the field' in Nature Biotechnology in 2009. A research group in Japan successfully inserted and expressed the *lea* (late embryogenesis abundant) gene for drought tolerance in kidney beans using sonication and vacuum infiltration. The major issues and hurdles preventing the development of this technology in beans fall under five major areas discussed below. In the U.S. there have been no reports on the development of GM beans in the scientific literature.

Major Issues related to GM beans are addressed under different subheadings:

- Technology Issues – formidable as beans are a recalcitrant species, not easily manipulated at the tissue culture level which is an essential step in gene insertion.
- Ownership – IP Issues for the Technology and Specific Genes (Transgenes) – cost of licensing technology
- Limited number of suitable genes for genetic insertion
- Regulatory Issues – cost of seeking government approval – deregulation issues
- Consumer Perception of GM bean products

Technology Issues:

- Thirty years ago scientists discovered that the pathogenic soil bacterium *Agrobacterium tumefaciens* is capable of inter-kingdom genetic transfer. The crown gall-causing bacterium can integrate transfer DNA (T-DNA) on the tumor-inducing (Ti) plasmid into the genomes of most crops. Since this discovery, *Agrobacterium*-mediated DNA transfer has been exploited to introduce transgenes into plants and to transform other organisms such as yeast, fungi and even human cells.
- Unlike soybeans, beans have been neglected as a biotech crop by the private sector. The limited acreage base of the crop and the diversity of market classes limit its attractiveness as a commodity for investigation. The majority of research advances in bean comes from the public sector.

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- Beans, similar to soybeans, are a recalcitrant crop not readily amenable to tissue culture and regeneration techniques that are a vital part of genetic modification protocols.
- The introduction of a foreign gene into beans requires technology protocols that a new whole bean plant can be regenerated from a single 'omnipotent' cell. Efficient regeneration systems have not been successfully developed for beans, but have been developed for related crops such as tepary bean, cowpea and pea.
- Efficient regeneration systems are generally genotype specific i.e. they do not work with the same level of efficiency for all crop varieties.
- Plant transformation systems using *Agrobacterium* (Agro) differ in efficiency between genotypes and different strains of Agro infect different varieties at varying levels of efficiency.
- Other transformation systems do exist and involve physical manipulation such as particle bombardment, sonication and direct DNA infusion. These systems suffer from a low level of efficiency, and offer no control over where the target gene is inserted. As a result there may be no gene expression as that region of the genome is not expressed. Other limitations can be transient expression of the transgene, or multiple copies may be inserted resulted in gene 'silencing' or lower levels of gene expression. Agro-mediated transformation is the preferred method as many of these problems can be avoided but it does require efficient regeneration system to produce genetic modified plants carrying the transgene.
- Beans are sensitive to Agro-mediated transformation but different strains of Agro need to be tested against different bean genotypes as there is specificity between interactions. Different chemicals need to be tested to enhance regeneration as the protocols for other related crops require media adjustments to successfully regenerate plants.
- The explant tissue used for regeneration can produce variable results. Preference is given to somatic embryogenesis or organogenesis producing cells from leaf or stem callus tissue rather than embryogenesis as the later produces multiple cells from the embryonic axis that may result in chimeric transgenics rather than a single transgenic event which would result in stable transformants.
- Bean plants have resisted attempts to successfully regenerate plants from individual cells. This is a formidable hurdle. Regeneration is possible from the embryonic axis but it has the limitation that many cells are involved and since transformation is targeted at single cells not multiple cells, transformed chimeric plants may result.

IP Issues:

- The decision to transform bean with a specific trait will depend on who owns the target gene. Permission from Monsanto would be required to use the GOX gene for glyphosate resistance or from Bayer CropScience for use of the *bar* gene controlling glufosinate resistance.
- Proof of concept may be granted but the commercial application of these technologies will require initial approval from both companies owning the target genes.
- Representatives from the Bean Industry would need to determine if any opposition to the use and application of this technology exists as the owner may wish to restrict its application to specific crops in a production area where they have a major financial investment.

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- Practical considerations regarding volunteer round-up ready crops that become a weed problem in next year's glyphosate resistant crops are easier to control in a rotation where glyphosate is not applied every crop year.
- Biolistic technology for use in bean transformation is also patented and would require license agreements.

Availability of GM Traits for Genetic Engineering:

- A clear need or opportunity has to be demonstrated to develop GM crops. Is the need production driven or consumer driven to increase consumption? Aside from insect and herbicide resistance, there is a paucity of useful traits available for genetic engineering. Most new traits may already be IP protected.
- Convenience traits
- Essential traits
- Consumer traits
- Novel traits - what other traits should be considered?

Regulation Issues:

- All field testing of GM crops is regulated through the USDA and is usually a straightforward notification process for current transgenes. If the transgene that is inserted is non conventional (example drug product) rather than current routine genes (insect or herbicide resistance), approval may not be forthright.
- If herbicide resistance is the targeted gene, EPA approval will be needed to apply that specific herbicide to the bean crop. Approval requires substantial testing and is very costly. Decisions on which organization will seek and pay for that approval will need to be made by the bean industry. Costs routinely exceed \$10m.
- All GM traits a/o products in beans would have to be deregulated in the U.S. before any GM beans could be produced or sold. Private industry would have to assume the cost of deregulation which could be substantial. Addressing the deregulation issue will be difficult given the segmented nature of the bean industry across states.

<http://www.nytimes.com/2010/05/15/opinion/15ronald.html?ref=opinion>

GM Summary:

- GM Beans: In order to maintain competitiveness of beans with other commodities, GM beans may need to be developed.
- Require Industry approval – and financial support – venture capital
- Establish different partnerships with private sector
- Regulation – require USDA and EPA approval
- Technology – viable reproducible transformation system has to be developed
- Routinely regenerate beans through tissue culture system that is not limited by genotypic specificity
- Preferred system to transform beans is using *Agrobacterium* rather than random particle bombardment methods that may be transient and may introduce multiple copies of the inserted gene.

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- Confirm stability of the trait by passing through number of seed generations from the initial T0 generation when expression was first detected to confirm expression in later T2 and T3 seed generations.
- Identify specific traits for insertion - herbicide resistance –bar gene for Liberty-link glufosinate resistance; GOX gene for glyphosate resistance [Roundup Ready®]; disease resistance – Oxo gene against white mold; drought tolerance – lea or Xerico genes; and nutritional enhancement – no gene action known.
- Specific traits, consider IP issue of ownership; technology transfer protocols used to insert gene.
- Owners may not support the expanded use of specific transgene in another crops – competition between commodities
- Public perception of trait or GM product, potential value of the trait in beans, acceptability, target – production or consumer.
- Regulation of GM bean, notification through USDA for field testing
- EPA approval needed if chemical pesticides need to be applied to the GM crop – costs estimates exceed \$10m.
- Deregulation of GM beans needs to be addressed before GM bean products arrive in the U.S.

Table 1. History of Bean Breeding Research at MSU

DECADE	MILESTONES IN BEAN BREEDING AT MICHIGAN STATE UNIVERSITY
1900's	<i>Establishment of bean breeding program at Michigan Agricultural Experiment Station, Michigan State College under the direction of Dr. Spragg</i>
1910's	<i>Release of Robust navy bean variety in 1915. Robust was a line selected yield, uniformity and resistance to virus from among a lot of commercial beans</i>
1920's	<i>Active program established in navy bean strain testing and breeding at MSU.</i>
1930's	<i>Release of Michelite navy bean variety in 1938. Michelite was the first bred variety released by MSU under direction of Dr. Down</i>
1940's	<i>Attempts to use X-rays to generate novel genetic variability useful in bean breeding.</i>
1950's	<i>Release of Sanilac navy bean in 1956 by Drs. Andersen, and Down. Sanilac was the first bush navy bean released by MSU</i>
1960's	<i>Release of series of early season bush navy bean varieties, Seaway, Gratiot, and Seafarer by Dr. Adams. Beans were destined for European markets via the St. Lawrence Seaway.</i>
1970's	<i>Release of Montcalm dark red kidney bean in 1974 by Drs. Adams and Saettler. Montcalm was the first halo blight resistant kidney bean which revitalized that industry in Northern Michigan</i>
1980's	<i>Class diversification into black and pinto beans. Release of first black, pinto, great northern bean varieties, Domino, Black Magic, Sierra and Alpine by MSU</i>
1990's	<i>Broad adoption of the full-season, high-yielding, upright short vine navy and black bean varieties by growers under direction of Dr. Kelly</i>
2000's	<i>Release of first small red bean by Dr. Hosfield. Class diversification continued with release of the first pink, otebo and soldier bean varieties by MSU</i>

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Table 2. List of Bean Varieties (by class) Released from Public Bean Breeding Programs

<u>Black(21):</u>	<u>Source</u>	<u>Kidney(34):</u>	<u>Source</u>	<u>Small Red(16):</u>	<u>Source</u>
Midnight	Cornell	RedKanner-LRK	Cornell	Common Red Mex	landrace
Black Knight	Cornell	Red Kloud -LRK	Cornell	Merlot	MSU
C49-242	Cornell	Red Kote-LRK	Cornell	Rufus	UI
Shiny Crow	CSU	Ruddy-LRK	Cornell	UI 229	UI
T-39	UCD	Wallace -LRK	Cornell	UI 259	UI
Jaguar	MSU	Montcalm-DRK	MSU	UI 34	UI
Raven	MSU	Red Hawk-DRK	MSU	UI 35	UI
Condor	MSU	Charlevoix-DRK	MSU	UI 36	UI
Zorro	MSU	Isles-DRK	MSU	UI-3	UI
Rhino, -115M	MSU	Chinook 2000LRK	MSU	USWA-9	UI
Black Magic	MSU	Mecosta-LRK	MSU	Big Bend	USDA
Domino	MSU	Isabella-LRK	MSU	LeBaron	USDA
Blackhawk	MSU	Chinook-LRK	MSU	NW 63	USDA
Phantom	MSU	Beluga-WK	MSU	UI 228	USDA
Eclipse	NDSU	CELRK-LRK	UCD	UI 37	USDA
UI 911	UI	California DRK	UCD	USRM-20	USDA
UI 906	UI	California LRK	UCD		
A55	USDA	Linden-LRK	UCD	Pinks (19)	<u>Source</u>
I9365-31	USDA	Badillo-LRK	UPR	Early pink	landrace
92BG-7	USDA	Kamiakin-LRK	USDA	Standard pink	landrace
ICB-10	USDA	K-42	USDA	Sedona	MSU
ICB-3	USDA	K-59	USDA	Sutter Pink	UCD
		K-407	USDA	Salinas	UCD
		Lisa-WK	USDA	Yolano	UCD
		USDK-CBB-15	USDA	UI-537	UI
		USDK-4	USDA	Rosada Nativa	UPR
		Fiero -DRK	USDA	Viva	USDA
		Royal Red-DRK	USDA	Roza	USDA
		Kardinal-LRK	USDA	Coulee	USDA
		Blush-LRK	USDA	Harold	USDA
		USLK-1	USDA	Gloria	USDA
		Silver Cloud-WK	USDA	UNS-117	USDA
		USWK-CBB-17	USDA	6R-42	USDA
		USWK-6	USDA	Victor	USDA
				USWA-61	USDA

Cornell=NY; CSU=CO; MSU= MI; NDSU=ND; UCD=CA; UI=ID; UNL=NE; UPR=PR; USDA=WA

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<u>Pinto (52):</u>	<u>Source</u>	<u>Pintos - continued</u>	<u>Source</u>	<u>Navy(24):</u>	<u>Source</u>
Bill Z	CSU	Burke	USDA	Aurora	Cornell
Olathe	CSU	USWA-20	USDA	Michelite	MSU
Montrose	CSU	TARS-VCI-4B	USDA	Sanilac	MSU
Arapaho	CSU	JM-126	USDA	Seafarer	MSU
Croissant	CSU	Columbia	USDA	Swan Valley	MSU
San Juan	CSU	Pindak	USDA	Neptune	MSU
Ouray	CSU	Nodak	USDA	C-20	MSU
Sierra	MSU	Holberg	USDA	Bunsi	MSU
Aztec	MSU	92US1006	USDA	Mayflower	MSU
Kodiak	MSU	ICB-12	USDA	Laker	MSU
Santa Fe	MSU	Quincy	USDA	Huron	MSU
Maverick	NDSU	USPT-ANT-1	USDA	Newport	MSU
Lariat	NDSU	USPT-CBB-5	USDA	Mackinac	MSU
Stampede	NDSU			Seahawk	MSU
ND-307	NDSU	<u>Great</u>		Norstar	NDSU
Frontier	NDSU	<u>Northern(29):</u>		Avalanche	NDSU
Hatton	NDSU	Matterhorn	MSU	ND88-106-04	NDSU
Luna	NMSU	Alpine	MSU	UI 137	UI
UI 111	UI	UI 59	UI	Verano	UPR
UI 114	UI	UI 1	UI	Morales	UPR
Common Pinto	UI	UI 31	UI	NW-395	USDA
Shoshone	UI	US1140	UI	Hyden	USDA
Kimberly	UI	Sawtooth	UI	USWA-48	USDA
SDIP-1	UI	UI 425	UI	USWA-50	USDA
UI 126	UI	UI 123	UI		
UI 129	UI	GN Harris	UNL		
UI 196	UI	GN Star	UNL	<u>Cranberry (13)</u>	
UI 320	UI	Jules	UNL	G122	USDA
ABCP-8	UNL	Tara	UNL	Capri	MSU
Chase	UNL	Startlight	UNL	Cardinal	MSU
ABCP-15	UNL	Emerson	UNL	Cranberry	landrace
ABCP-17	UNL	Weihing	UNL	Crimson	USDA
PT47	UPR	ABC-Weihing	UNL	Taylor Hort	MSU
Othello	USDA	Coyne	UNL	UI 50	UI
NW590	USDA	BelNeb-RR 1, 2	UNL	UI 51	UI
NW 410	USDA	BelMiNeb- RMR-1, 5	UNL	UI 686	UI
PT7-2	USDA	GN#1Sel27	UNL	USCR-7	USDA
USPT-WM-1	USDA	JM-24	USDA	USCR-9	USDA
USPT-CBB-1	USDA	USWA-12	USDA	USCR-CBB-20	USDA
USPT-CBB-3	USDA	USWA-13	USDA	Bellagio	MSU

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Conclusions: Bean breeding will continue to advance as the study of genomics and its application to beans is realized. Field breeding and selection will continue to play an important role in development of future bean varieties. Breeders will be able to use new SNP marker platform to more efficiently select for specific traits as these tools are developed as part of the BeanCAP. Additional information on nutritional traits in beans is forthcoming and some of these traits will be included in the list of traits targeted for improvement. GM beans will become a reality in the future and should be more acceptable to consumers if breeders use genetic engineering to target nutritional rather than production traits. Bean breeders will need to maintain flexibility to add new traits to the list for improvement if industry deems them important for producers and consumers alike. Historically, bean breeding programs have been located at land-grant institutions in the major production states. This might change as universities are unable to fill positions due to limited state funding for applied research as the emphasis of competitive federal funding is on basic research.

Glossary/Terminology:

Alleles: Alternate forms of the same gene. In normal diploid organisms such as bean there are normally only two alleles in a particular plant, although multiple alleles controlling a trait may be available in bean germplasm. Disease resistance genes in plants may have multiple alleles.

Backcross breeding: Breeding system where each generation the hybrid F1 is crossed back to commercial parent known as recurrent parent to add a new trait to the existing variety.

Cleistogamous: Flowering pattern where the flower is already self-pollinated before it opens. This occurs in perfect flowers (those with both male anthers and female style) like beans.

Genomics: The science of gene structure and arrangement within biological organisms.

GxE: Known as the Genotype x Environmental Interaction. Not all traits are expressed similarly across environments. For example a high yielding bean may produce less yield in certain environments. GxE explains that portion of a trait that interacts differentially with the environment.

Heritability: The portion of a complex trait that is under genetic control and can be manipulated through breeding. The other portion is under environmental control and varies by year or location.

Linkage: Arrangement of genes along a linear chromosome. Genes next to each other on the chromosome are physically linked. Those on separate chromosomes or on separate arms of the same chromosome are not linked.

MAS: Marker-assisted selection, tool used in plant breeding where a gene of interest is linked to a genetic marker – usually a molecular marker. By selecting for the marker, breeders are indirectly selecting for the gene of interest.

Pedigree breeding: System of breeding self pollinated crops where selection is practiced each generation of inbreeding to fix different traits in a final pure line variety.

Polymorphism: Multiple forms of molecular markers – expressed as bands of different molecular weight on an agarose gel. In order to be valuable markers must be polymorphic. Markers that exhibit only one molecular weight band are said to be monomorphic and provide limited genetic information.

QTL: Quantitative Trait Loci is based on statistical probability that a portion of a quantitative trait resides at that location (loci) in the bean genome.

Segregation: Genetic ratios observed among traits following cross pollination between two diverse parents.

Syntenry: Similar gene order observed along the chromosomes of two related species.

Transformation: the process of inserting a foreign gene into a plant is known as plant transformation and the gene once inserted and functional (expressed) is known as a transgene.

Transgene: Gene from another species or organism transformed into plant through genetic engineering.

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Appendix of Web Sites by Topic:

Production data by class and region

http://www.usdrybeans.com/home/default_usr.aspx

<http://www.ers.usda.gov/Briefing/drybeans/PDFs/DBnOutlook.pdf>.

<http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1394>.

Principles of Mendelian genetics, Meiosis, and plant breeding

<http://www.dnalc.org/resources/animations/index.html>

<http://www.youtube.com/watch?v=iCL6d0OwKt8&feature=related>

<http://cuke.hort.ncsu.edu/gpb/>

<http://theagricos.com/plant-breeding/>

http://www.youtube.com/watch?v=JlaABbNPISg&feature=player_embedded

<http://www.ndsu.edu/pubweb/~mcclean/plsc431/mendel/mendel1.htm>

<http://vcell.ndsu.edu/animations/>

<http://www.hrt.msu.edu/pbgp/Links/plantbreedingintro.html>

List of major bean breeding programs in the U.S. – includes information on varieties

<http://www.css.msu.edu/bean/>

<http://www.ag.ndsu.nodak.edu/plantsci/breeding/drybean/>

<http://www.nebraskadrybean.com/research20.htm>

http://www.colostate.edu/programs/wcrc/pubs/research_outreach/fndbeans.htm

<http://www.kimberly.uidaho.edu/beans/>

USDA-ARS programs focus on different aspects of bean improvement.

<http://www.ars.usda.gov/pandp/people/people.htm?personid=3848>

<http://www.ars.usda.gov/Research/docs.htm?docid=4337>

<http://www.ars.usda.gov/is/AR/archive/jun06/beans0606.htm>

<http://www.ars.usda.gov/pandp/people/people.htm?personid=40697>

<http://www.ars.usda.gov/is/AR/archive/may10/May-June2010.pdf>

Germplasm and Plant Variety Protection

<http://www.ars-grin.gov/>.

<http://www.ams.usda.gov/AMSV1.0/getfile?dDocName=STELDEV3002796>

http://www.ciat.cgiar.org/AboutUs/Documents/synthesis_bean_program.pdf

Diseases

<http://www.whitemoldresearch.com/HTML/drybeans.cfm>.

<http://www.css.msu.edu/bic/ResearchTechniques.cfm>

<http://legume.ipmpipe.org/cgi-bin/sbr/public.cgi>

Variety Trial Results, Production Issues

<http://www.maes.msu.edu/ressta/saginawvalley/bean1.html>

http://www.css.msu.edu/VarietyTrials/DryBean_HomePage.html

<http://cuke.hort.ncsu.edu/cucurbit/wehner/vegcult/beandry.html>

<http://www.waaesd.org/exotic-germplasm-conversion-and-breeding-common-bean>