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RECENT DEVELOPMENTS OF APPLIED GENETICS IN THE FIELD OF AGRICULTURE

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The objective of this report is to show how new ideas culminating from years of "theoretical" exploration in the field of genetics can benefit present day agriculture. The science of genetics has taught us that genes have been mutating, segregating, and re-arranging themselves independently since evolutionary time began. Genes have been primarily responsible for the evolution of plant and animal life to the present level.

Careful research by geneticists, during the last 50 years, has shown how the "power of a gene" can, first of all, be understood, and secondly, be harnessed to do important work in agriculture.

Four general fields of research in genetics appear to offer definite promise in the improvement of our economic crops.

I. ARTIFICIALLY INDUCED MUTATIONS

The possibility of increasing the spontaneous mutation rates of genes has occupied the attention of plant breeders since the turn of the century. Some research in the 1920's used X-rays in an attempt to induce gene changes artificially. But it was not until 1927 that H. J. Muller, working with the fruit fly, *Drosophila melanogaster,* showed clearly how the natural mutation rate could be increased by applying radiant energy in the form of X-rays. He predicted that the availability of artificially induced, mutant races would offer tremendous possibilities for the plant and animal breeder.

In 1928, Stadler working independently with barley, *Hordeum vulgare* L., confirmed the fact that mutation rates could be altered in plants as well as in animals.

Since the 1920's, a great deal of the radiation work for the improvement of plants has been conducted by plant breeders in Sweden, namely, Nilsson-Ehle, Gustafsson, and MacKey. Their work has recently been reviewed by Gustafsson (1951). The Swedish workers isolated several mutations of barley which were characterized by very dense heads and stiff straws. These so-named "erectoides" mutants outyielded the original Golden variety by 1.3% in tests conducted over a 10-year period. From an agronomic point of view, the significant contribution made by radiation of barley was the addition of a stiffer stalk, which would tend to prevent lodging and subsequently bolster yields.

In this country, Gregory (1954) screened 975,000 progenies of Xradiated peanuts, *Arachis hypogea* L., var. Virginia bunch. He found several mutant strains which were higher in yield and had better resistance to the common leaf spot disease, *Cercospora arachidicola*.

K. J. Frey (1954), at Iowa State, has been successful in X-raying seeds of oats, *Avena sativa* L. Some of the mutant oats were shorter and had stiffer straws than the Huron variety. Four others "headed" a week earlier, a few showed resistance to races 7 and 8 of oat stem rust, *Puccinia graminis avenae* Erikss. and E. Henn. Some of the mutant oat strains yielded 60 bushels per acre with a test-weight as high as 31 pounds per bushel. The standard check variety, Huron, in this same test yielded 30 bushels per acre with a test-weight of 21 pounds per bushel.

Konzak (1954) working with Mohawk oats, variety CI4327, reported that in 1953 he isolated 73 mutant plants which showed resistance to oat stem rust 7a, following an artificial inoculation of the pathogen. Seed from which these progenies grew had been previously placed in the thermal column of the nuclear reactor at the Brookhaven National Laboratory. Mutations were induced by the ionizing effect of thermal neutrons on seeds exposed 4 and 8 hours to a flux of approximately 4.6 x 10^8 thermal neutrons /cm.^{2}/sec. The resistance to stem rust which was obtained appears to be controlled by a single dominant gene.

Another interesting project at Brookhaven has been the construction of a continuous gamma radiation field. A central source of radioactive cobalt 60, which can be lowered underground when not in use, irradiated plants grown in circular rows around the source of the gamma-rays. Singleton (1955) learned that best results were obtained by bringing the plants into the field for short periods of time, and then removing them to a "portable", non-radiated field. In one experiment, corn plants were brought in for one day and placed on the 6-meter circle where they were exposed to 1,300 roentgens. A much higher rate of endosperm mutations was obtained than with lower doses per day under chronic radiation. Singleton and his co-workers also found that the optimum time for irradiating corn plants was in a period after meiosis and just prior to the shedding of pollen.

Three examples of ionizing mutagenic agents have been described: X-rays, thermal neutrons, and gamma rays. The nature of changes induced are about the same for all three. Many of the mutations are recessive, and are for the most part deleterious. Some sources of radiation may induce more changes per unit of energy than others. Caldecott *et al.* (1954) has found that Himalaya barley seeds were much more uniformly affected by thermal neutrons than by X-radiation. The highest mutation frequency obtained with thermal neutrons, without causing a high degree of sterility, was two times that obtained with 20,000 roentgens. It is the ionization effect of X-rays, gamma rays, and thermal neutrons which tends to break complex chromosome molecules. As a result, parts of chromosomes are broken off or translocated. Because of this, some gene loci are deleted or altered, so that different genetic expressions result.

Stakman (1954) pointed out that probably one of the best services radiation could render would be to break chromosome linkages between beneficial and deleterious genes. He cited the situation in *Triticum* where a variety, recently produced in Mexico, is highly rust resistant. However, the section of the stem just below the terminal inflorescence is very weak and limber. The ''limber neck'' gene or genes are linked with the genes for rust resistance and will complicate breeding in wheat until this linkage can be broken.

Mutation rates can also be increased by the application of ultraviolet rays, mustard gas, manganous chloride, and internal trace elements such as C^{14} and P^{32}. In addition to these agents, there are genic and extragenic units, which exert a specific type of control on the action of the gene with which they become associated. These units are capable of transposition from one location in the chromosome to another. One example of this, is the dissociation-activator system (Ds-Ac) of McClintock (1953). Ds is responsible for the modification of genic expression. The Ac component determines when changes by the Ds will occur. Other systems inducing mutations by genes and chromosome systems are being discovered; in time these may provide the plant breeder with new material for improved breeding stock.

II. POLYPLOIDY IN PLANTS

A useful phenomenon which occurs in plants is the multiplication of the basic chromosome number from a $2n$ or normal diploid condition to a 4, 5, 6, or $8n$, etc. polyploid condition. In general, polyploids are characterized by an increase in height of plant, in thicker stems, broader leaves, larger fruiting bodies, larger stomata, and larger pollen grains. There are exceptions, however, and many polyploids will be found whose parts are reduced in size.

1. *Autopolyploids* are formed by an increase in the basic number of chromosomes of a single species. This can occur in nature or can be induced artificially by an agent such as the plant alkaloid colchicine. Muntzing (1951) reported that tetraploid varieties of red clover, *Trifolium pratense* L., are commercially available in Europe. In yield tests conducted at various locations in Sweden these autotetraploid clovers out-yielded the normal diploids 11 out of 15 times. In performance trials on winter rye, *Secale cereale* L., Muntzing found that autopolyploids out-yielded normal diploid rye by 55%. Furthermore, the flour from tetraploid rye had better baking qualities. Autodiploidy has proven most successful in plants in which seed yield is not an important factor, because plants with this type of polyploidy are often semi-sterile. Where root, leaf, flower, and fruit size, as well as vitamin content, are important, autopolyploidy has distinct advantages.

One interesting application of autopolyploidy is in the development by Japanese breeders of a triploid, seedless watermelon. First a normal diploid melon, *Citrullus vulgaris* Schrad., is doubled to the tetraploid form. The tetraploids are then crossed with diploids. The resulting triploid plants will be sterile and seedless. Consequently, fertile diploid plants must be in the same field to provide normal pollen for stimulation of ovule development.

2. The second type of polyploidy is called *allopolyploidy* or *amphidiploidy.* These forms are often the result of interspecific crosses. The two chromosome sets are doubled artificially to produce a new fertile variety. Allopolyploidy has occurred in nature during the course of evolution in some of our economic plants such as upland cotton, *Gossypium hirsutum* L. and wheat, *Triticum vulgare* Vill. One useful application of amphidiploidy is in the improvement of existing species of cotton. This was recognized by Beasley (1942) who crossed Arizona wild cotton, *Gossypium thurberi* Tod., with Asiatic wild cotton, *G. arboreum* L. He then doubled, with colchicine, the $(n = 13) \times (n = 13)$ cross to obtain a fertile amphidiploid $(2n = 52)$ cotton. The amphidiploid cotton could then be crossed readily with American upland cotton $(2n = 52)$. This technique enabled cotton breeders to introduce genes for such things as disease resistance and good fiber qualities into existing commercially important species.

A similar technique is used in the improvement of wheat (Sears, 1947 and 1948). By crossing a related diploid, *Aegilops speltoides* Tausch, with tetraploid wheat, *Triticum dicoccoides* Körn, a triploid results and is sterile. However, by doubling the chromosome complement, it becomes fertile and will cross readily with the common hexaploid wheats. Thus, valuable rust resistance factors found in *Aegilops* spp. can be introduced in the common variety of wheat.

III. SELECTIVE BREEDING

Plant breeders have in recent years attempted to improve old, mass selection techniques by developing systems for concentrating more favorable genotypes within a given population. These up-graded populations would then provide a source for elite inbred lines which upon subsequent re-combination express heterosis in a much higher degree.

1. Stadler, in 1944, proposed *gamete selection.* His technique with maize, *Zea mays* L., involves a maximum selection of superior gametes by crossing uniform inbred lines with open-pollinated varieties. The hybrids are then self-pollinated and at the same time out-crossed to a uniform tester strain whose genetic make-up will not mask the hereditary differences of the strains being tested. The inbred line is also outcrossed to this same tester. Both types of tester crosses are then compared for yielding ability. If certain *variety x tester* combinations significantly outyield the *inbred x tester* cross, those gametes are considered superior to the inbred line, and warrant further inbreeding (Brown, 1953).

2. Hull (1945) developed a system of recurrent selection for specific combining ability based on the theory that over-dominance $(AA < Aa > aa)$ is largely responsible for heterosis. One or two three-generation cycling periods are required for building up a favorable gene population which has been recurrently selected against a tester

inbred line or single cross. Inbreds developed from this synthetic population of favorable genes should combine well with the tester lines or single cross. Sprague and Brimhall (1950) have found recurrent selection effective in selection for high oil content in corn kernels. Jenkins *et al.* (1954) successfully isolated lines resistant to *Helminthosporium turcicum* leaf blight. Johnson (1952) has been able to improve sweet clover, *Melilotus alba* Desr., by one cycling period of recurrent selection.

3. An additional refinement of the technique of mass selection has been termed *reciprocal recurrent selection* Comstock *et al.* (1949). Two sources of genetically diverse material are used for selection—open-pollinated A and synthetic B, for example. Recurrent selections from A will be test-crossed with B as a tester, and those of B crossed with A as a tester. In this way favorable genes from both composite sources, A and B, will be accumulated, and lines derived from them should recombine with maximum vigor.

4. A new method of selection is the isolation of superior gametes through the monoploid method of producing homozygous diploid lines. Chase (1949, 1953) successfully developed new inbred lines of corn by this system; they perform as well as or better than lines developed by the conventional method of inbreeding. One advantage of this system of selection is that it enables one to screen a large mass of material for elite genotypes. Once superior gametes are recognized by an adequate testing procedure, they can then be utilized immediately in recombination with other homozygous diploid lines or conventional inbreds.

IV. Cytoplasmic Male Sterility

Control of normal pollen production by the cytoplasm was first observed in maize by Rhoades (1933). Factors producing this type of sterility are transmitted independently from one generation to the next through the cytoplasm of the female egg cell. Whether or not the cytoplasmic factors for sterility will manifest complete control over pollen production is dependent on the nuclear genes. The first commercial application of cytoplasmic pollen sterility was made in hybrid production of the onion, *Allium cepa* L., H. A. Jones *et al.* (1937, 1947). Today, however, the most widespread use of cytoplasmic male sterility is in the production of hybrid seed corn. It was first pointed out by D. F. Jones and Everett (1949) that a great deal of detasseling in corn production could be eliminated if a completely pollen-sterile single cross is used in place of the normal, fertile seed parent. Actually, both fertile and sterile counterparts of a hybrid can be produced in the same field. Blending at harvest time is accomplished by picking a seed row of the fertile single cross, and then a seed row of the male sterile single cross. This insures an adequate mixture of fertile, pollen-shedding plants. Since all female flowers are fertile, a normal seed set will result. The control of pollen production by nuclear genes was described by Jones (1950) and Rogers and Edwardson (1952). Certain inbred lines of corn contain genetic factors for pollen restoration which will overcome pollen abortion by the cytoplasm. These restorer genes promote normal pollen grain development. The use of restorer lines in hybrid seed corn production would enable a hybrid to be produced without blending in the field.

General improvement of hybrid corn can be expected from the use of cytoplasmic male sterility for the following reasons: 1. inbred line increase and single-cross foundation seed production require intensive supervision; 2. the addition of restorer factors in the male single-cross may bring in other beneficial genes which are linked with the restorer genes; and 3. the decrease in the amount of detasseling necessary for commercial seed production will reduce injury to the female plants normally encountered through removal of leaves and infection by plant diseases.

In addition to maize and onion, hybrid seed of other plants such as the carrot, *Daucus carota* L., sugar beet, *Beta vulgaris (saccharifera)* L., and petunia, *Petunia violacea* Lindl, is being produced with a cytosterile female parent.

Recently, Stephens and Holland (1954) announced that in sorghum, *Sorghum vulgare* Pers., hybrid grain production is commercially possible through the use of cytoplasmic male sterility. Increases in yields up to 100% have already been reported from widespread strip tests and yield trials. The increased yield of hybrid sorghum, together with continued drought in the corn growing areas and acreage restrictions, within a very short period of time should double the present yield from the 11 million acres of sorghum grown.

It would be difficult at this time to predict which one of these four fields of genetic research will benefit agriculture most. Evidence has been presented which shows that efficient, peacetime applications of atomic energy can greatly increase the mutation rate. With this higher frequency of gene mutations in plants, the greater will be the chances of locating beneficial changes. It has also been shown that by means of polyploid species and improved selection techniques, disease resistance and additional yield factors can be incorporated in our economic crops. The discovery of male-sterile factors in the cytoplasm and of fertility restoration genes in the nucleus has increased the possibilities for hybrid production in many of our cultivated species. With pollen sterility, tedious emasculation of perfect-flower plants, such as the onion, is no longer necessary for cross pollination. To screen many genera of the plant kingdom for cytosterile strains will become the plant breeders next important challenge.

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