Crop Breeding

Sakshi Sharma¹, Ms. Shweta Tyagi², Sangmeshwar Yewange³, Dr.Abhimanyu Kumar jha⁴

^{1,2,4} Department of Biotechnology, Faculty of Life Science, Institute of applied medicines and research, Ghaziabad, India

³Latur College of Pharmacy Hasegaon, Tq. Ausa, Dist. Latur- 413512, Maharashtra, India

Abstract - Crop breeding can be broadly defined as alterations caused in Crop as a result of their use by humans, ranging from unintentional changes resulting from the advent of agriculture to the application of molecular tools for precision breeding. The vast diversity of breeding methods can be simplified into three categories: (i) Crop breeding based on observed variation by selection of Crop based on natural variants appearing in nature or within traditional varieties; (ii) Crop breeding based on controlled mating by selection of plants presenting recombination of desirable genes from different parents; and (iii Crop breeding based on monitored recombination by selection of specific genes or marker profiles, using molecular tools for tracking within-genome variation. The continuous application of traditional breeding methods in a given species could lead to the narrowing of the gene pool from which cultivars are drawn, rendering crops vulnerable to biotic and abiotic stresses and hampering future progress. Several methods have been devised for introducing exotic variation into elite germplasm without undesirable effects. Cases in rice are given to illustrate the potential and limitations of different breeding approaches.

Index Terms - Crop Breeding, botany, breeding, research, cultivated, Crop, Crop genetics, Cropphysiology, Crop pathology, plant growth, Crop development.

INTRODUCTION

Crop breeding is the art and science of improving important agricultural plants for the benefit of humankind. Crop breeders work to make our food, fiber, forage, and industrial crops more productive and nutritious. Crops provide for an expanding global population with increasing dietary expectations. Environmental protection is also improved by the work of crop breeders.

Plant breeding has been practiced by farmers since the dawn of agriculture, as they selected plants for larger seeds, more tasty fruits, and other valuable traits. Today, both farmers and scientists work to breed plants.

DIFFERENT KINDS OF CROP BREEDING

- 1. Backcrossing or introgression breeding
 - Crop breeders sometimes use a process called backcrossing. A plant that has the desirable traitlet's say mildew resistance-is crossed with a plant that doesn't have that trait but is desirable in all other traits. There is a quality control step to make sure that the only change to the original variety is the desired trait. For example, a high-yielding pea can be crossed with a mildew-resistant pea. The next generation plant is called the progeny. All progeny that are still mildew resistant are then crossed to their high-yielding parent. This is repeated a few more times, always crossing back to the high-yielding parent, and selecting the mildew-resistant progeny. This process ensures the next generation is in most ways similar to the high-yielding parent while adding the mildewresistant quality from the other parent.
- 2. Inbreeding

Depending on the species, some plants may be fertilized by themselves. This is done to produce an inbred variety, which is exactly the same generation after generation. Because it preserves the original traits, it is useful in three ways: for research; as new, true-breeding cultivars; and as the parents of hybrids.

3. Hybrid breeding

In this situation, two different inbred varieties are crossed to produce an offspring with stable characteristics and hybrid vigor, where the offspring is much more productive than either parent.

4. Mutation breeding

Naturally occurring genetic mutations exist throughout the world. If these random examples are found and seen as an improvement, they can be used to create new varieties. Alternatively, mutations can be artificially encouraged by exposing plants to chemicals or radiation.

5. Molecular marker-assisted selection

> This uses classical, backcrossing, or inbreeding and hybridization methods, with an important difference. Instead of selecting desirable plants based on the way they look or grow, breeders select plants after confirming the information on the genes the plants inherited from their parents. Just like having a map to an unfamiliar city, this takes some of the guesswork out of breeding. Researchers can confirm the gene is present, not just assume it is, before they move forward with breeding the plant.

Genetic engineering 6.

> Engineers who design bridges or skyscrapers insert strong building design into their plans. Similarly, modern genetics techniques can insert desirable traits into plants. The resulting plants are called transgenic or genetically modified organisms (GMOs).

7. Gene editing

These cutting-edge genetic techniques, including CRISPR-Cas9, enable breeders to modify specific genes directly. It targets very specific plant characteristics with razor-like precision. Classification of crop plants based on mode of pollination and mode of reproduction.

Mode of pollination	Examples of crop plants
and reproduction	
Self-Pollinated Crops	Rice, Wheat, Barley, Oats,
	Chickpea, Pea, Cowpea, Lentil,
	Green gram, Black gram, Soybean,
	Common bean, Moth bean,
	Linseed, Sesame, Khesari, Sun
	hemp, Chillies, Brinjal, Tomato,
Cross Pollinated Crops	Okra, Peanut, Potato, etc.
	Corn, Pearl millet, Rye, Alfalfa,
	Radish, Cabbage, Sunflower, Sugar
	beet, Castor, Red clover, White
	clover, Safflower, Spinach, Onion,
	Garlic, Turnip, Squash,
Often Cross-Pollinated	Muskmelon, Watermelon,
Crops	Cucumber, Pumpkin, Kenaf, Oil

palm, Carrot, Coconut, Papaya, Sugarcane, Coffee, Cocoa, Tea, Apple, Pears, Peaches, Cherries, Almond Strawberries, grapes, Pineapple, Banana, Cashew, Irish, Cassava, Taro, Rubber, etc. Sorghum, Cotton, Triticale, Pigeon pea, Tobacco.

BREEDING METHODS IN CROP PLANTS

SELF POLLINATED CROPS

Mass selection

In mass selection, seeds are collected from (usually a few dozen to a few hundred) desirable appearing individuals in a population, and the next generation is sown from the stock of mixed seed. This procedure, sometimes referred to as phenotypic selection, is based on how each individual looks. Mass selection has been used widely to improve old "land" varieties, varieties that have been passed down from one generation of farmers to the next over long periods.

An alternative approach that has no doubt been practiced for thousands of years is simply to eliminate undesirable types by destroying them in the field. The results are similar whether superior plants are saved, or inferior plants are eliminated: seeds of the better plants become the planting stock for the next season.

A modern refinement of mass selection is to harvest the best plants separately and to grow and compare their progenies. The poorer progenies are destroyed, and the seeds of the remainder are harvested. It should be noted that selection is now based not solely on the appearance of the parent plants but also on the appearance and performance of their progeny. Progeny selection is usually more effective than phenotypic selection when dealing with quantitative characters of low heritability. It should be noted, however, that progeny testing requires an extra generation; hence gain per cycle of selection must be double that of simple phenotypic selection to achieve the same rate of gain per unit time.

Mass selection, with or without progeny test, is perhaps the simplest and least expensive of plantbreeding procedures. It finds wide use in the breeding of certain forage species, which are not important enough economically to justify more detailed attention.

Pure-line selection

Pure-line selection generally involves three more or less distinct steps: (1) numerous superior appearing plants are selected from a genetically variable population; (2) progenies of the individual plant selections are grown and evaluated by simple observation, frequently over a period of several years; and (3) when selection can no longer be made on the basis of observation alone, extensive trials are undertaken, involving careful measurements to determine whether the remaining selections are superior in yielding ability and other aspects of performance.

Any progeny superior to an existing variety is then released as a new "pure-line" variety. Much of the success of this method during the early 1900s depended on the existence of genetically variable land varieties that were waiting to be exploited. They provided a rich source of superior pure-line varieties, some of which are still represented among commercial varieties. In recent years the pure-line method as outlined above has decreased in importance in the breeding of major cultivated species; however, the method is still widely used with the less important species that have not yet been heavily selected.

A variation of the pure-line selection method that dates back centuries is the selection of single-chance variants, mutations, or "sports" in the original variety. A very large number of varieties that differ from the original strain in characteristics such as colour, lack of thorns or barbs, dwarfness, and disease resistance have originated in this fashion.

Hybridization

During the 20th century planned hybridization between carefully selected parents has become dominant in the breeding of self-pollinated species. The object of hybridization is to combine desirable genes found in two or more different varieties and to produce pure-breeding progeny superior in many respects to the parental types.

Genes, however, are always in the company of other genes in a collection called a genotype. The plant breeder's problem is largely one of efficiently managing the enormous numbers of genotypes that occur in the generations following hybridization. As an example of the power of hybridization in creating variability, a cross between hypothetical wheat varieties differing by only 21 genes is capable of producing more than 10,000,000,000 different genotypes in the second generation. At spacing normally used by farmers, more than 50,000,000 acres would be required to grow a population large enough to permit every genotype to occur in its expected frequency. While the great majority of these secondgeneration genotypes are hybrid (heterozygous) for one or more traits, it is statistically possible that 2,097,152 different pure-breeding (homozygous) genotypes can occur, each potentially a new pure-line variety. These numbers illustrate the importance of efficient techniques in managing hybrid populations, for which purpose the pedigree procedure is most widely used.

Pedigree breeding starts with the crossing of two genotypes, each of which have one or more desirable characters lacked by the other. If the two original parents do not provide all of the desired characters, a third parent can be included by crossing it to one of the hybrid progenies of the first generation (F1). In the pedigree method superior types are selected in successive generations, and a record is maintained of parent–progeny relationships.

The F2 generation (progeny of the crossing of two F1 individuals) affords the first opportunity for selection in pedigree programs. In this generation the emphasis is on the elimination of individuals carrying undesirable major genes. In the succeeding generations the hybrid condition gives way to pure breeding as a result of natural self-pollination, and families derived from different F2 plants begin to display their unique character. Usually, one or two superior plants are selected within each superior family in these generations. By the F5 generation the pure-breeding condition (homozygosity) is extensive, and emphasis shifts almost entirely to selection between families. The pedigree record is useful in making these eliminations. At this stage each selected family is usually harvested in mass to obtain the larger amounts of seed needed to evaluate families for quantitative characters. This evaluation is usually carried out in plots grown under conditions that simulate commercial planting practice as closely as possible. When the number of families has been reduced to manageable proportions by visual selection, usually by the F7 or F8 generation, precise evaluation for performance and quality begins. The final evaluation of promising strains involves (1) observation, usually in a number of years and

647

locations, to detect weaknesses that may not have appeared previously; (2) precise yield testing; and (3) quality testing. Many plants breeders test for five years at five representative locations before releasing a new variety for commercial production.

The bulk-population method of breeding differs from the pedigree method primarily in the handling of generations following hybridization. The F2 generation is sown at normal commercial planting rates in a large plot. At maturity, the crop is harvested in mass, and the seeds are used to establish the next generation in a similar plot. No record of ancestry is kept. During the period of bulk propagation natural selection tends to eliminate plants having poor survival value. Two types of artificial selection also are often applied: (1) destruction of plants that carry undesirable major genes and (2) mass techniques such as harvesting when only part of the seeds is mature to select for early maturing plants or the use of screens to select for increased seed size. Single plant selections are then made and evaluated in the same way as in the pedigree method of breeding. The chief advantage of the bulk population method is that it allows the breeder to handle very large numbers of individuals inexpensively.

Often an outstanding variety can be improved by transferring to it some specific desirable character that it lacks. This can be accomplished by first crossing a plant of the superior variety to a plant of the donor variety, which carries the trait in question, and then mating the progeny back to a plant having the genotype of the superior parent. This process is called backcrossing. After five or six backcrosses the progeny will be hybrid for the character being transferred but like the superior parent for all other genes. Selfing the last backcross generation, coupled with selection, will give some progeny pure breeding for the genes being transferred. The advantages of the backcross method are its rapidity, the small number of plants required, and the predictability of the outcome. A serious disadvantage is that the procedure diminishes the occurrence of chance combinations of genes, which sometimes leads to striking improvements in performance.

Hybrid varieties

The development of hybrid varieties differs from hybridization. The F1 hybrid of crosses between different genotypes is often much more vigorous than its parents. This hybrid vigour, or heterosis, can be manifested in many ways, including increased rate of growth, greater uniformity, earlier flowering, and increased yield, the last being of greatest importance in agriculture.

CROSS POLLINATED CROPS

The most important methods of breeding crosspollinated species are (1) mass selection; (2) development of hybrid varieties; and (3) development of synthetic varieties. Since cross-pollinated species are naturally hybrid (heterozygous) for many traits and lose vigour as they become purebred (homozygous), a goal of each of these breeding methods is to preserve or restore heterozygosity.

Mass selection

Mass selection in cross-pollinated species takes the same form as in self-pollinated species; i.e., a large number of superior appearing plants are selected and harvested in bulk and the seed used to produce the next generation. Mass selection has proved to be very effective in improving qualitative characters, and applied over many generations, it is also capable of improving quantitative characters, including yield, despite the low heritability of such characters. Mass selection has long been a major method of breeding cross-pollinated species, especially in the economically less important species.

Hybrid varieties

The outstanding example of the exploitation of hybrid vigour through the use of F1 hybrid varieties has been with corn (maize). The production of a hybrid corn variety involves three steps: (1) the selection of superior plants; (2) selfing for several generations to produce a series of inbred lines, which although different from each other are each pure-breeding and highly uniform; and (3) crossing selected inbred lines. During the inbreeding process the vigour of the lines decreases drastically, usually to less than half that of field-pollinated varieties. Vigour is restored, however, when any two unrelated inbred lines are crossed, and in some cases the F1 hybrids between inbred lines are much superior to open-pollinated varieties. An important consequence of the homozygosity of the inbred lines is that the hybrid between any two inbreds will always be the same. Once the inbreds that give the

best hybrids have been identified, any desired amount of hybrid seed can be produced.

Pollination in corn (maize) is by wind, which blows pollen from the tassels to the styles (silks) that protrude from the tops of the ears. Thus, controlled cross-pollination on a field scale can be accomplished economically by interplanting two or three rows of the seed parent inbred with one row of the pollinator inbred and detasselling the former before it sheds pollen. In practice most hybrid corn is produced from "double crosses," in which four inbred lines are first crossed in pairs (A \times B and C \times D) and then the two F1 hybrids are crossed again $(A \times B) \times (C \times D)$. The double-cross procedure has the advantage that the commercial F1 seed is produced on the highly productive single cross $A \times B$ rather than on a pooryielding inbred, thus reducing seed costs. In recent years cytoplasmic male sterility, described earlier, has been used to eliminate detasselling of the seed parent, thus providing further economies in producing hybrid seed.

Much of the hybrid vigour exhibited by F1 hybrid varieties is lost in the next generation. Consequently, seed from hybrid varieties is not used for planting stock but the farmer purchases new seed each year from seed companies.

Perhaps no other development in the biological sciences has had greater impact on increasing the quantity of food supplies available to the world's population than has the development of hybrid corn (maize). Hybrid varieties in other crops, made possible through the use of male sterility, have also been dramatically successful and it seems likely that use of hybrid varieties will continue to expand in the future. Synthetic varieties

A synthetic variety is developed by intercrossing a number of genotypes of known superior combining ability-i.e., genotypes that are known to give superior hybrid performance when crossed in all combinations. (By contrast, a variety developed by mass selection is made up of genotypes bulked together without having undergone preliminary testing determine their performance in hybrid to combination.) Synthetic varieties are known for their hybrid vigour and for their ability to produce usable seed for succeeding seasons. Because of these advantages, synthetic varieties have become increasingly favoured in the growing of many species,

such as the forage crops, in which expense prohibits the development or use of hybrid varieties.

MUTATION BREEDING

Physical Mutagens

Physical mutagens include various types of radiation, viz X-rays, gamma rays, alpha particles, beta particles, fast and thermal (slow) neutrons and ultraviolet rays. A brief description of these mutagens is presented below:

Commonly used physical mutagens (radiations), their properties and mode of action.

Type of	Main properties			
Radiation				
X – rays	S.I., penetrating and non-particulate			
Gamma rays	S.I., very penetrating and Non-particulate			
Alpha Particles	D.I., particulate, less penetrating and positively charged.			
Beta Rays Particles	S.I., particulate, more penetrating than alpha particles and negatively charged.			
Fast and Thermal	D.I., particulate, neutral particles,			
Neutrons	highly penetrating.			
Ultraviolet Rays	Non-ionizing, low penetrating			

Note: particulate refers to particle emitting property DI = Densely ionizing, SI = Sparsely ionizing.

X-rays

X-rays were first discovered by Roentgen in 1895. The wavelengths of X-rays vary from 10-11 to 10-7. They are sparsely ionizing and highly penetrating. They are generated in X-rays machines. X-rays can break chromosomes and produce all types of mutations in nucleotides, viz. addition, deletion, inversion, transposition, transitions and transversions. X-rays were first used by Muller in 1927 for induction of mutations in Drosophila. In plants, Stadler in 1928 first used X-rays for induction of mutations in barley.

Gamma rays

Gamma rays have shorter wavelength than X-rays and are more penetrating than gamma rays. They are generated from radioactive decay of some elements like 14C, 60Co, radium etc. Of these, cobalt 60 is commonly used for the production of Gamma rays. Gamma rays cause chromosomal and gene mutations like X-rays.

Description on Gamma chamber

It is a compact, self-contained irradiation unit offering an irradiation volume of approximately 1000CC with adequate protection and sealed so that, the radiation leakage outside the unit is well below the maximum permissible dose level. No extra shield is required in the laboratory. The chamber at TNAU, Coimbatore was installed during October 1972. The main unit essentially consists of three parts.

a) Source cage, b) Biological shield for source, c) central shaft with irradiation chamber.

- a. Source cage: holds the irradiation source in an angular cylinder, the co-axial hole in the center of the cage provides space for irradiation chamber. The cage is designed to hold 18 to 20 pencils containing cobalt 60, in the form of pellets. The design of the source cage optimize to provide maximum uniformity of dose rate in the site of irradiation chamber.
- b. Biological shield: It consists of two parts I) Main outer shield ii) Removable plug. The radiation source is housed in the main outer shield which is designed to reduce the radiation on the outer surface or to a value less than maximum permissible limit for laboratories. The inner removable plug is a cylindrical lead plug with a co-axial hole and act as a guide for the shaft. The removable plug permits easy loading and unloading of radiation source in the main field.
- c. Central shaft with irradiation chamber: Central shaft is a solid lead cylinder at the center of which sample chamber is located. The size of the sample chamber is 14.3cm high with 10 cm diameter. The purpose of central lead is to provide shielding during upward and downward movement of the sample chamber.

Sample chamber and control panel

The sample chamber is raised or lowered by a wire rope, using system of pulling by a rotating drum. The drum is rotated by an electric motor or self-locking rotating gear. The circuit which controls the movement includes a synchronised motor, operator, timer and upward and downward switches. Provision is made for manual as well as automatic operation and the mode of operation is decided by panel switch. Instructions for Operation

- a. Switch on the power key. Push the 'up'. The chamber will come up.
- b. Open the chamber and place the materials to be irradiated inside and close the chamber.
- c. Set the irradiation time on the timer
- d. Put the timer switch on auto. Push the 'down' switch, at the end of the prescribed time, the sample automatically comes out. If the irradiation is to be terminated, prior to the prescribed time, put the switch in the manual operation and pres the 'up' switch.

CHEMICAL MUTAGENS

Procedure for chemical mutagenesis

The chemical mutagens can be divided into four groups, viz. 1) alkylating agents, 2) base analogues, 3) acridine dyes, and 4) others. A brief description of some commonly used chemicals of these groups is presented below.

Some commonly used chemical mutagens and their mode of action

Grou	up	of	Name of	chemical	Mode of activ	on
muta	agens	8				
1. Alkylating Agents		Ethyl	methane	← →		
		Sulphonate		AT	GC	
		Methyl Methane		Transitions		
		Sulphona	te	Transitions		
1.	Bas	e	Ethyl	Ethane	← →	
Analogues		Sulphona	te	GC	AT	
		Ethylene Imines		Transitions		
	1.	Acridine	5 Bro	moUracil	Transitions	
		Dyes	2 Amino	purine	←→	
			Acriflavi	1,	AT	GC
	1.	Others	Proflavin		Transitions	
					←→	
			Nitrous	Acid	AT	GC
		Hydroxyl	amine	Transitions		
		Sodium A	Azide	Deletion,	additior	
				and frame sh	ifts.	
					←→	
					AT	GC
					Transitions	
					← →	
					GC	AT
					Transitions	
					Transitions	

The speed of hydrolysis of the chemical mutagens is usually measured by the half-life of the chemicals. Half-life is the time required for disappearance of the half of the initial amount of active reaction agent. The following table gives the half-life in hours at different temperatures.

Chemicals		Temperature				
		200C	30OC	37OC		
MMS	(hours)	68	20	9.1		
EMS		93	26	10.4		
DES		3.3	1	-		
NMU		-	35	-		
NEU		-	84	-		

In the case of DES the mutagenic solution should be changed at every half an hour to get good results. Half-life is the function of temperature and pH for a particular compound.

One should be extremely careful in handling alkylating agents since most of them are carcinogenic. Especially for ethylene imine, it should be handled under aerated conditions. EMS though not dangerous, it should not be pipetted out by mouth. Besides the alkylating agents, we are also having chemical mutagens like, Base analogues, Acridine dyes, Antibiotics and other miscellaneous chemicals.

Treatment of seeds with mutagenic chemicals:

Materials required: conical flask, beaker, pipette, glass rods, measuring cylinder, stopwatch, distilled water and phosphate buffer.

Method: Mutagenic chemical is diluted to the required concentration by using distilled water. To prepare the molar concentration of DES, the method is

Molecular weight x a.i. (purity percentage)/ Specific gravity (active ingredient)

Eg. DES = $\begin{array}{ccc} 154 & 100 \\ ---- & x & ---- \\ a. & 18 & 99 \end{array}$ = 131 CC.

131 CC dissolved in one liter will give 1 molar solution.

Seeds have to be soaked in the distilled water for different hours depending upon the seeds, to initiate biochemical reactions. The chemical action is found to be affected by the frequency and spectrum of mutagen depending upon the stage of cell division, during the process of germination. If the chemical treatment is synchronized with DNA synthesis stage (G1, S and G2) then we can get better results.

The presoaked seeds are taken in a flask and chemical is added. Usually, the quantity of the chemical is ten times the volume of seeds. Intermittent shaking should be given to ensure uniform exposure of the chemicals. The chemical should be drained after the treatment time is over. The seeds should be washed thoroughly in running tap water, immediately for not less than 30 minutes. After washing, the seeds should be dried in between the filter paper folds. Seeds are to be arranged in germination tray with equal spacing. Trays are kept in a controlled environment of temperature and humidity. Periodical observation on germination up to 10-15 days is needed. From the germination percentage, we can assess the LD50 dose.

CONCLUSION

Many agricultural crops of economic importance are vegetatively propagated. This work was done to elaborate more on vegetatively propagated crops, importance and developing new cultivars of vegetative propagated crops. Vegetative propagation aids to fix favorable combinations of important traits, very specific chemical compositions, superior genetic variance interactions and high levels of heterozygosity.

Breeding vegetative propagated crops involve few genetic crossing and genetic combination by sexual reproduction. Vegetatively propagated crops are best suited for maintaining hybrid vigour because once a desired hybrid has been produced; there are fewer chances of losing it. Breeding by mutation enables to get improved characters in plant like tolerance to environment pressure, plant shape, changes in oil content. disease resistant in plant and protein quality.

REFERENCES

- Kasha, K.J. 1974. Hapoids in higher plants. (Ed. Kasha, K.J.) University of Guelph, Guelph, Canada
- [2] RaKow, G. 1990. personal communication. Ag.Canada Research Branch, Saskatoon, Canada.
- [3] Keller, W.A. 1990. personal communication. From Manbo Seeds, Holesy, Denmark.r
- [4] Shugar, L., 1990. W. G. Thompson & Sons Ltd., Box 130, Blenheim, Ontario, Canada.

- [5] Kasha, K.J., 1990. personal communication.
- [6] Wernsman, E.A. 1990. personal communication. From Chili Tabacos.
- [7] Nielson, M.T., Legg, P.D. and Collins, G.B. 1988. Crop.Sci 29, 242.
- [8] Bollich, C.N. & Magill, C. 1990. personal communication.
- [9] Buyser, J., et al. 1987, Plant Breeding 98, 53–56.
- [10] Genovisi, A.D. 1990. In. Biotechnology in Agriculture and Forestry: Haploids in Crop Improvement. I. (Y.P.S. BAJAJ, ed) Vol 12. pp. 176–203. Springer Verlag.
- [11] 11.Dunwell, J.M. 1976. Env.and Exp. Botany 16, 109–118.
- [12] Kasha, K.J. and Reinbergs, E. 1979. Proceedings 2nd International Haploidy Symposium pp. 215-230.
- [13] Dunwell, J.M. 1983. Annals of Botany 48, 535– 542.
- [14] Shannon, P.R., et al. Plant Cell Tiss Org Cult 4, 271–280.
- [15] Petolina, J.F., et al. 1988. TheorAppl Genet 76, 157–159.
- [16] Keller, W.A., and K. C. Armstrong. 1978. Zeitschrift Fur Pflanzenzuchtung 80, 100-108.
- [17] Pechan, P.M. and Keller, W. A. 1989. In Vitro Cell & Dev Biol 25, 1073–1074
- [18] Dunwell, J.M., et al. 1985 J. Exp. Bot. 36, 1–11.