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Review on; Seed Genetic Purity for Quality Seed Production

Yeshiwas Sendekie
Ethiopian Institute of Agricultural Research, Pawe Agricultural Research Center, Pawe, Ethiopia
E-mail address: yeshiwassendekie @ gmail.com

***Abstract*—** *Production and productivity of the crops are decreasing from time to time due to lack of improved varieties and other management factors. Improved varieties are known for their excellent genetic, physical, and physiological purity. Hybrid seed production is highly contaminated by crossed pollen from another variety or self-pollination. The development of hybrid varieties requires high genetic quality seeds. Quality seeds are efficient to use agricultural inputs such as fertilizers and irrigation to produce the desired quality and amount of seed. The direct contribution of quality seed alone can increase production by 25% wisely combined with other inputs. However, during the seed production process, genetic purity of seed is lost due to developmental variation, mechanical mixtures, natural crossing, genetic drift, the influence of disease, mutations, and adverse climatic conditions. The extent of genetic deterioration is higher in cross-pollinated crops than self-pollinated ones. Yield loss after self-seed production of self-pollinated crops are not serious up to certain generations such that the yield reductions amount to about after one year, 96% after two years, 90% after three years and 83% after nine years, but, in hybrid seed production, it will be null. These problems could be minimized through crop rotation, isolation distance, field inspection, seed certification, and seed legislation by applying genetic purity control techniques (i.e. morphological, chemical and molecular).*

***Keywords*—** *Genetic deterioration; Genetic engineering (GE); Genetic purity.*

I. INTRODUCTION

Seed is the basic input in agriculture and plays a crucial role in boosting up the production, productivity and economy of the country. To meet the growing needs of the population and enhancing production and productivity, timely availability of quality seed at reasonable price to the farming community is desirable (Sudipta, 2012). In the case of cross-pollinated crops, such as the use of hybrid varieties, it is one of alternatives to maximize production and productivities (Awaludin *et al*., 2013**)**.

Quality seeds have the ability for efficient utilization of the inputs such as fertilizers and irrigation so as to attain food security (Mirza, 2015). It is very important not only to get higher yield but also to get high monetary returns. Quality seed alone can increase the production by 25% wisely combined with other inputs can increase yield levels (Deo *et al*., 2003).

Quality seed production in general affected by the genetic purity, physiological quality, and the presence of weed seeds, seed borne diseases and other materials. Genetic purity is the percentage of contamination by seeds or genetic materials of other varieties or species. The genetic purity of any commercial agricultural product propagated by seed begins with the purity of the seed planted (Bradford, 2006). During seed production process due to certain reasons the genetic purity of the seed may be lost, this is said to be deterioration of a particular crop variety through developmental variation, mechanical mixtures, natural crossing, genetic drift, influence of disease and mutations (Parimala, 2013). Nowadays, the introduction and commercial cultivation of GM crops on any scale would immediately create problems for farmers and the rest of the supply chain because of contamination of non-GM and organic crops (Freeze, 2012).

These problems of genetic deterioration could be identified and maintained through molecular techniques, traditional approach to genetic purity testing, chemical methods, control plot method, quarantine and other cultural method (Venkata, 2014**).** However, replicated field observations are time-consuming, expensive and unreliable. Morphology methods like, plant height, seed size and color cannot provide strict information on the grain quality of specific genetic attributes to pest or herbicide resistance into varieties. Biochemical assays and Isozymes have been commonly used and can distinguish varieties within several species (i.e., .in maize (*Zea mays* L.). Newer DNA-based technologies such as molecular markers are more recently developed methods having great potential for enhancing purity assessment of hybrids. Thus, can allows clear differentiation and faster identification of varieties so as to (Smith and Register, 1998). This review describes the importance of seed genetic quality, factors that contribute to the genetic deterioration and the important method to maintain and control the genetic purity.

II. SEED QUALITY

Seed is any plant part which is utilized for commercial multiplication of a crop. It includes stem (sugar cane) modified stem (onion) and true seed itself (cereals, pulses, oil crops etc). In strict sense, seed is a fertilized (matured) ovule consisting of embryonic plant, stored food and seed coat which is viable and has the capacity to germinate. It is a structure developed by flowering plants after fertilization (Sudipta, 2012). Seed carries the genetic material naturally and/or incorporated through breeding that could be transferred from generation to generation in the production process. Seed quality describes; genetic purity, physiological purity, physical purity and seed health (i.e. weed seed, diseased seed, inert material etc) (Bishaw *et al*. (2007); Venkata, 2014).

Genetic purity is the percentage of contamination by seeds or genetic materials of other varieties or species (Kent, 2006). Genetic purity is the most important factor in plant breeding and seed producer, which refers to trueness to type, or the degree of contamination of seeds caused by undesired genetic varieties or species. It may consist different attributes such as plant type, duration of growth cycle, seed color and shape seedling vigor, insect resistance, disease resistance, cold season productivity, high seed yield, non-seed shattering and specific grain or seed qualities (PIRSA, 2013). Genetic purity can determine whether the seed can adapt to local conditions, and often influence farmer and market demand. While some varieties may be affected differently by storage conditions than others, storage conditions will not affect the actual genetic composition of the seed (Walsh, 2014).

Seed genetic purity mostly associated with sexually propagated plants; it is more severe in cross-pollinated than self-pollinated crops. The success of hybrid seed production is dependent on the genetic purity of parental lines. Both outcrossing and the inadvertent mixing of seed can compromise seed quality. Therefore, genetic purity maintenance and tests are critical tools for seed producers and plant breeders.

A. *Importance of Quality Seed for Sustainable Agricultural Production*

Seeds are the foundation of agriculture. Seeds are vital input in crop production and plant breeding. Good quality seeds have the potential for efficient utilization of agricultural inputs that leads to increased economic return. Without the use of quality seed, the investments incurred on inputs i.e. fertilizer, pesticide and irrigation will not pay dividend which ought to be realized. To meet the growing needs of the population and enhancing the production and productivity of the country, timely availability of quality seed at a reasonable price to the farming community is desirable (Sudipta, 2012). Mirza (2015) also confirmed that quality seeds have the ability for efficient utilization of inputs such as fertilizers and irrigation. Quality seed is very important not only to higher yield but also to get high monetary returns. Quality seed alone can increase the production by 25% wisely combined with other inputs significantly increase yield levels (Deo *et al*., 2003).

The development of hybrid varieties needs to be supported by the availability of genetically pure seeds. Genetic purity is the most dominant factor for successful seed production of maize hybrid. In producing hybrid seeds, it is frequently contaminated by crossed pollen from another variety or the occurrence of self-pollination (Awaludin, *et al*., 2013**)**.

Farmers' seed production is developed through experimentation and experience acquired over a long period of time. Their practices are often well adapted to local conditions. A study on local bean seed quality by showed that in 11 of the 13 reviewed cases farmers' seed quality was at least as good as seeds from the formal sector. A similar study on cereal seed quality in a number of countries in Western Asia and Northern Africa showed similar results. Not all farmers are equally good seed producers (Almekinders, 2000).

III. Genetic Purity Characteristics

According to IRRI (2013) and genetic purity described by its physical, chemical and crop attributes.

A. *Physical Attributes*

Genetically pure seeds of varieties are characterized by specific traits such as the length and width, shape, size, color and aroma. It is quantified by physical measurements using the naked eye and colorimeter, and smell tests both in the nasal and the machine (IRRI, 2013).

B. *Chemical Analysis*

Varieties have at least one distinct character that could be tested by various chemicals to distinguish them. Amylase content, alkali digestion color, gel consistency and brown rice protein help to differentiate varieties.

C. *Crop Factors*

The crop characteristics that are genetically determined include plant height, time to maturity, plant color and plant growth habit are the genetic purity attributes. These characters may show slight variations in harsher environmental growing conditions.

IV. Genetic Purity Deterioration

Genetic purity deterioration is permanent reduction either in the genetic or agronomic value of a given crop. Deterioration in seed quality may begin at storage and any point in the plant's development stage from sowing till threshing. Seed quality depends upon the physical conditions that the mother plant is exposed to different environmental conditions during the growth stages, as well as harvesting, processing, storage and planting. Temperature, nutrients and other environmental factors also affect seed development and influence seed quality (IRRI. 2013).

According to Bradford (2006), the genetic purity of the seed planted must equal or exceed the final product purity standard required, as purity generally decreases with each subsequent generation of propagation. On the other hand, it is virtually impossible to assure that no off-type plants or pollens are present in the seed production field and all handling equipment and storage facilities are completely free of contamination, so commercial planting seed is seldom 100% pure.

Yield loss after self-seed production of self-pollinated plants by farmers is not serious up to certain generations such that the yield reductions amount to about after one year, 96% after two years, 90% after three years and 83% after nine years (Atilaw, *et al*., 2011). In the case of hybrid variety, it may lead to below half within one year (Awaludin, *et al*. 2013).

4.1. Causes of Genetic Purity Deterioration

The main objective of seed production is to produce good quality and genetically pure seeds that could be used for next-generation seed production. But, during seed production due to certain reasons, the genetic purity of the seed may be lost, this is said to be adeterioration of a particular crop variety

(Parimala, 2013). According to Kadam (1942), some of the reasons for genetic purity deterioration are.

A. Developmental variation

It occurs when a seed variety is grown out of its area of adaptations (i.e., different environment, different soil, fertility conditions and altitudes) for several consecutive generations developmental variation may occur to adapt these variable environmental conditions.

B. Mechanical mixtures

This kind of deterioration may take place at any stage of development from sowing to storage. It may arise due to the occurrence of volunteer plant seeds, use of the same planter for two different varieties of the same crop, growing different varieties adjacent to each other, using unclean threshing floor and poor storage and packing conditions.

C. Natural crossing

This is most common in the case of sexually propagated crops. The extent of crossing depends upon the breeding system of the crop (i.e. whether it is self-pollinated, cross-pollinated or often cross-pollinated), isolation distance and its pollinating agent.

D. Genetic drift

This kind of deterioration may happen when a seed crop is grown in a large area and only a small quantity of seed is conserved for sowing next year, this will not be represented in the next generation.

E. Influence of disease

The occurrence of newer races of pests and diseases may lead the new crop variety to develop tolerance and resistance mechanism, thus developmental variation causes deterioration. Some virus, fungi and bacteria, even cause genetic deterioration on vegetative propagated.

F. Mutations

It is of meager importance as the occurrence of spontaneous mutations is very low. Adverse agro-climatic conditions during the field and unfavorable storage condition may cause the genetic deterioration of the variety through addition, chromosomal aberrations and mutations (Sudipta, 2012).

G. The techniques of the plant breeder

In the release of new varieties, serious instability may occur in varieties owing to cytogenetic irregularities in the form of improper assessments. Varieties released without completing their segregating for resistance and susceptibility to diseases or other factors can cause significant deterioration of varieties.

H. Genetic engineering

Genetically modified (GM) crops are crop plants created for human or animal consumption, which have been modified in the laboratory to enhance desired traits or improved nutritional content. Nowadays, the introduction and commercial cultivation of GM crops on any scale would immediately create problems for farmers and the rest of the supply chain because of the contamination of non-GM and organic crops (Freeze, 2012). In developed countries, cross-pollination of GM seeds onto non-GM crops is also a concern to seed producers, particularly those farmers that certify their crops as non-GM crops or organic crops (Price and Cotter, 2014).

There is an evidence showed the existence of cross-pollination between GM and non-GM (Rieben et al., 2011) and, Price and Cotter (2014) elaborated that the presence of genetic contamination occurred on rice and maize crops on their review. Genetically engineered (GE) varieties have created additional issues for seed genetic purity, particularly for seed producers seeking to meet organic marketing standards or who are engaged in international trade (Kent, 2006). A study on wheat indicated that, higher cross-pollination occurred in GM wheat varieties than in non-GM varieties (Rieben *et al*., 2011).

Besides its importance, it has toxicity (using similar methods to those used for conventional foods), gene flow to neighboring crops and to related wild species, reduced crop genetic diversity, may favor new pathogens, tendency to provoke any allergic reaction, Stability of the inserted gene and unintended effects of the gene insertion (Jeff and Katherine, 2006).

4.2. Techniques to Maintain Genetic Purity

a. Crop rotation

The land selected for seed production should be free from varieties of the same crop species for at least one or two years of the same variety. The right previous cropping is necessary to avoid genetic contamination in seed production. Similarly, in cereal seed production, the previous cropping with other cereal crops such as wheat, oats, barley or rye should be avoided, because a seed crop of wheat, for instance, is very difficult to purify by rouging if contaminated with an excessive mixture of other cereals (Atilaw *et al*., 2011). Seed production which is free from genetically engineered seeds should not be grown in a field that has previously grown genetically engineered crops of the same species within the past 3 to 5 years to ensure that volunteer plants will not be present (Bradford, 2006). For non-genetically engineered seed production 2 years of rotation is enough to maintain genetic purity.

b. Isolation distance

It is the distance between the seed crops and other varieties of the same crop located in the adjacent area which helps to maintain the genetic purity of the seeds by preventing pollination from unwanted pollen in the case of cross-pollinated and often cross-pollinated species to prevent and to avoid mechanical mixture and the chance of cross-pollination in self-pollinated species. Isolation distance is based on the pollination behavior, pollinating agent, flying capacity of the pollen, stages of seed crop and nature of variety or hybrid. Maintaining the recommended isolation distance avoids cross pollination and crossing of genes. Two types of isolation are followed; the first is space isolation (planting distance) and the second is time isolation (difference in the time of sowing (Parimala, 2013).

According to Atilaw (2011) if the crop is self-pollinated often 2-3 meters around the edges of the field are adequate to prevent taking in any plants from the neighboring field during harvest. Cross-pollinated crops require longer distance than

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self-pollinated crops. If there is no possibility of getting the required isolation distance, it would be advisable to plant the seed crop at a different time, so that the seed crop will not be flowering when other fields of the same crops are shedding pollen.

TABLE 1: Minimum isolation distance of some field crops for genetic purity maintenance.

No.	crops	Isolation distance (in meter)		No.	crops	Isolation distance (in meter)	
		Foundation seed	Certified seed			Foundation seed	Certified seed
Self-Pollinated Crops				Cross -Pollinated Crops			
1	Paddy rice	3	3	1	Maize	400	200
2	Wheat	3	3	2	Pearl millet (Bajra) Hybrid	1000	200
3	Ragi	3	3	3	sunflower	400	
4	Soybean	3	3	4	Chillies	400	100
5	Groundnut	3	3	5	Potato	-	100
6	Blackgram and Greengram	10	-	6	Brinjal	200	100
7	Peas	20	10	7	Carrot	1,000	400-800
Often Cross -Pollinated Crops				8	Rapeseed	400	200
1	Cotton	50	30	9	mustard	400	200
2	Sesame	100	50				

Source; Parimala (2013).

c. Field inspection

Field inspection helps to minimize the genetic contamination, out-crossing, and transmission of seed borne diseases that cause the irreversible loss of genetic purity. During inspection rouging should be done. Rouging is the removal of off-types and diseased plants from the seed field before or after flowering (Malhotra and Vashishtha, 2017). It is used to avoid genetic contamination, outcrossing, and transmission of seed borne diseases that causes genetic deterioration. In self-pollinated crops rouging should be continuous, while in cross pollinated crops rouging should be done before anthesis and it should be done in both seed parent and pollinator rows (Atilaw *et al*., 2011).

Seed crops may be inspected frequently during the growing season. It is best to conduct inspection three times to allow the best opportunity to assess varietal identity and purity (i.e. Before flowering, at flowering and seed setting and Before harvesting) (Malhotra and Vashi0shtha, 2017 and Parimala, 2013).

V. Genetic Purity Control

a. Seed Certification

Seed certification helps to evaluate maintain and make available high-quality seeds and propagating materials of notified kind and varieties to seed producers through specific seed evaluation standards (Charles *et al*., 2010). Thus, grown and distributed as to ensure genetic purity. Seed certification is also designed to achieve prescribed evaluation standards. During certification process the evaluation parameter vary from crop to crop. However, certified seed crops must pass both field inspection and laboratory analysis.

TABLE 2. Genetic purity standards for evaluation

Two and three loci off types	Maximum of 0.5% (1 per 200 plants)
Variants	Maximum of 0.5% (1 per 200 plants)
Adventitious presence	Maximum of 0.1% at 95% Confidence (None found in 3,000)

Source: Charles Brown, (2015).

b. Seed Legislation

Both the seed trader and farming communities require protection against fraud, negligence or accident regarding seed quality. Laws can regulate the quality and marketing of seed, and can authorize the establishment of an organization with powers to develop rules for enforcement and implementation of the law. The ultimate aim is to ensure good quality standards and protect both consumer and producer from inferior seed. Legislation also allows advantage to be taken of the work of plant breeders and various crop improvement agencies (Charles *et al.*, 2010). This is very important for the regulation of GMO crop production to minimize the risk of crossing with undesired plant.

VI. Genetic Purity Testing

a. Morphological Methods

It is possible to evaluate the genetic purity on seed, on seedlings, in the lab or greenhouse, in the field. Plant habit Head color, Shape of shoulder. However, a very limited number of discriminatory traits can be observed directly on seed or seedlings, a limited number can be assessed on plants (Enrico, 2010). Morphology methods like, plant height, seed size and color cannot provide strict information on the grain quality of specific genetic attributes to pest or herbicide resistance into varieties (Smith and Register, 1998). Many of the morphological traits possess multigenic expression which is altered by environmental factors. These limitations are overcome by rapid and reliable methods of varietal identification and genetic purity testing and the best alterative way to speed up the testing procedure is to use chemical tests which have not been attempted to group chilli genotypes so far (Padma *et al*., 2015).

b. Control Plot Method

Control plot tests are used to monitor the identity and purity of a variety (hybrid or non-hybrid) at various stages in the seed multiplication program.

Pre-control is applied to variety verification of early generation seed, i.e. Pre-basic and Basic seed. Pre-control is a

very important component of a seed multiplication and certification program because of its ability to identify varietal identity and varietal purity insufficiencies at an early stage, before they become a major widespread problem. The pre-control is very reliable and for many species the only tool for the assessment of varietal identity (OECD, 2012).

Post-control is applied to variety verification of certified seed class which is not further multiplied. Post-control tests are nevertheless valuable, because they monitor how efficient the seed production process has been in maintaining varietal purity and identify ways in which the system might be improved (OECD, 2012).

c. Chemical Methods

Testing genetic purity using Chemical test like; Phenol, Ferrous sulphate, Fluorescence test, 2,4-D soak test, Peroxidase test, coper sulphat-ammonia, Potassium hydroxide (KOH), Gossypal, Potassium acid (HCI), Hydrogen peroxide (H2O2), Sodium hydroxide (NaOH), Potassium dichromate test, Lugol's solution, hydro chloric acid (HCl) and DDT (Elias *et al*., 2012; Venkata, 2014 and Padma *et al*., 2015). Some chemical tests rely on the presence of specific enzymes (e.g., peroxidase) or fluorescence chemical compounds within the seeds of different varieties. Other tests would require addition of chemical compounds (e.g., potassium hydroxide and hydrochloric acid) into the seeds to highlight seed features.

Mohamed (2011) used phenol test to differentiate wheat varieties and he concluded that It is the quickest method which produced distinct differences among varieties. It is a quick test for cultivar identification based on color reaction between phenol solution and seed coat (pericarp). It can be used for wheat, barley, oat, ryegrass and bluegrass. On the other hand, peroxidase test was used in soybean varieties differentiation. Buttery and Buzzell (1968) separated soybean cultivar into two groups based on the presence of either low or high seed coat peroxidase activity. Similarly, Muthuraj *et al*. (1999) and Elias *et al*. (2012), used peroxidase test to check varietal purity in soybean. peroxidase test also effective for differentiating the hybrids from their respective parents in cotton (Agrawal *et al*., 1988). According to Padma *et al* (2015) experiment on chili genotypes identification, they identified Potassium hydroxide and ferrous sulphate solutions to be useful for identification of female parent of chili hybrid and sodium hydroxide test was useful to differentiate chili genotype. They also identified the phenol and modified phenol tests did not stain different chili genotypes rendering all genotypes undistinguishable and grouping was not possible. Ryegrass, oat Fluorescence test were applied on different crops to improve seed quality such as, in soybean (Silvio *et al*., 2009).

Chemical tests for genetic purity are advantageous due to quick, don't need technical expertise or training, inexpensive to conduct, not required sophisticated equipment, it permits detection of percentage admixture of other type and its results are usually distinct and easily interpretable.

d. Herbicide or Insect Tolerance Tests

Genetic purity evaluation can also include screening for transgene (GM) contamination. Corn and beets, for example, are increasingly tested for the presence of transgenes. Utilizes sensitive, sophisticated, and powerful technologies to detect the presence of transgenic events and crop varieties. With superior expertise and high-throughput capability, we are able to offer genetic purity test services with high quality, short turnaround at competitive rates. Seeds or seedlings are exposed to particular herbicides or insects; and their performances are monitored for the evaluation of their tolerance (Elias *et al*., 2012).

e. Molecular Markers

DNA technology has great potential for purity assessment of hybrids and transgenic plants. Any genetically determined traits (morphological, biochemical, molecular) can be distinguished among genotypes. Molecular markers detect variation directly at DNA level (i.e. the character is expressed in all developmental stages at a known position in the genome and sometimes in genes of interest). Most common molecular markers RAPD (Random Amplification of Polymorphic DNA), restriction fragment length polymorphism (RFLP), SCAR (Sequence Characterized Amplified Region), Different molecular markers, Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) sequence-tagged microsatellite (STMS) (Vecchia *et al*., 1998; Nandakumar *et al*., 2004; Enrico N. 2010 and Bora *et al*., 2016) can be utilized for the identification of plant varieties. Assessment of genetic purity of hybrid seed based on fertility restorer gene linked to the co-dominant STMS marker was found to be more reliable than the non-linked one (Garg *et al*., 2006). The markers used for assessing hybrid seed purity should be selected carefully after taking into consideration the varieties grown in an adjacent field that can serve as potential pollen donors (Yashitola *et a*l., 2002).

RAPD marker is a molecular genetic technique have been applied to plant cultivar identification in the past decade by developing molecular markers that detect differences in DNA sequences between cultivars. Highly specific marker profiles commonly known as DNA fingerprinting can be developed for each cultivar and used for its identification. Identifying breeding lines and determining hybrid purity is major requirements in plant breeding and quality seed production (Reddy, 2014). Rice hybrids can be identified using RAPD markers by counting the number of bands (Santhy *et al*., 2003). Jang *et al*., (2004) found that the SCAR method was superior to RAPD markers because of its simplicity in the analysis of bands and pellet painting can be done with SCAE markers, eliminating the need for electrophoresis.

Genetic purity of three F_1 chili hybrids was determined using two molecular marker techniques RAPD and ISSR. RAPD analysis successfully detected all three F_1 hybridity, while ISSR detected only two. This was due to the RAPD marker system producing a greater number of markers than the ISSR system (Mongkolporn *et al*., 2004). Nandakumar *et al*., 2004 and Bora *et al*., 2016, used sequence tagged microsatellite sites (STMS) markers for fingerprinting of

hybrids, assessing variation within parental lines and testing the genetic purity of hybrid seed lot in rice and they highlighted the importance of STMS markers in maintaining the genetic purity of the parental lines. SSR markers are powerful molecular markers capable of detecting genetic purity in maize hybrids (Daniel *et al*., 2012).

f. Biochemical and Imaging Technique for Analysis (BITA)

Isozyme analysis can be used for determination of genetic purity. However, this method is limited due to many factors affecting isozyme expression, including development of plant tissue and the environment. Less loci and restricted polymorphism may also affect the utility of these markers (Yan, 2013).

Electrophoresis of proteins showed promising results in genetic purity determination of hybrids and parental lines. The presence or absence of a specific band was much useful for differentiating the highest from the lowest parents in restoring ability (Galal *et al*., 2014**)**. Different research was conducted to identify a varietal difference through electrophoresis of proteins (Singh *et al., 2006).* Similarly, Wang *et al*., (1994) identified lentil varieties. And also, cultivar identification conducted using blotting of seed proteins from isoelectrically focused gels (McDonald, 1991).

VII. Conclusion and Recommendation

Improved seeds are characterized by their high genetic, physiological, physical and health quality. These qualities deteriorated through time during the production process by many factors including cross-pollination with transgenic plants. So, to keep the genetic purity of seeds, choice of simple and economical method or a combination of two or more quality control methods makes them accurate. The presence of genetic deterioration needs to be tested to produce good quality seed for the subsequent generation seed production. Chemical methods, molecular techniques for genetic purity identification and biochemical and imaging technique for analysis could be used to test genetic quality in the crop production process. Finally, seed quality evaluation is important. Therefore, development of simple, economical, applicable and easily available seed quality testing protocol is very vital for seed producer and plant breeders in general.

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