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Methods for Seed Varietal Identification for Agricultural Resilience

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Abstract: Seed varietal identification is a critical procedure in modern agriculture that has a big impact on crop production results and sustainability. This article examines different approaches to seed variety identification, highlighting the importance of seed varieties in maintaining crop quality, increasing productivity, and satisfying a range of agricultural demands. Conventional techniques, such as the Distinctness, Uniformity, and Stability (DUS) test and the Grow-Out Test (GOT), rely on morphological traits. Chemical techniques, such as the Peroxidase and Phenol Tests, provide accurate and timely results. High precision is achieved in differentiating closely related types using molecular techniques like Polymerase Chain Reaction (PCR) and biochemical techniques like electrophoretic analysis. Maintaining the genetic purity, legitimacy, and quality of seed supply is critical for regulatory compliance, market trust, and selective breeding. These identification procedures play a major role in ensuring these qualities. By employing these methods, farmers and researchers can make informed decisions that support sustainable agricultural practices and boost overall productivity.

Keywords: Genetic purity, Grow-out test (GOT), Seed varietal identification, Selective breeding, Sustainable agriculture

Introduction

In today's agriculture, seeds are fundamental because they have a major impact on crop production outcomes and sustainability. In agricultural systems, distinguishing between several seed kinds is a basic procedure that fulfils multiple vital functions (Azizi *et al*., 2021). Selective breeding procedures enable the development of improved plant types while also ensuring the authenticity and purity of seed supplies (MacLachlan *et al*., 2021). Furthermore, maintaining the reliability and dependability of agricultural products on the market requires

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meticulous adherence to regulatory criteria on seed quality and varietal purity. This paper will emphasize the critical role these methods play in ensuring crop quality, enhancing productivity, and meeting the diverse needs of agriculture across different contexts and environments.

What is Seed Varietal Identification?

The approach to distinguish different varieties or types of seeds within a plant species is known as seed varietal identification, which is of prime importance in agriculture. This identification is essential for maintaining crop purity, assisting breeding projects, adhering to regulations, and assuring market confidence (Mueller & Flachs, 2022). Farmers and researchers can enhance crop quality, make well-informed decisions, and support sustainable agricultural practices by correctly identifying seed varieties (Cooke, 2020).

Methods of Varietal Identification

Traditionally, the grow-out test (GOT) is used to assess the genetic purity of varieties. It is based on the evaluation of morphological and floral characteristics known as "descriptors" in plants that have reached maturity. There were two main reasons why varietal tests were relatively easy in the past: (1) there were fewer varieties, and (2) the differences between types were typically larger. Seed analysts needed newer, more dependable methods of differentiating between varieties in light of the success of contemporary plant breeding, the ensuing variety boom, and the emergence of several closely related kinds (Korir *et al*., 2013).

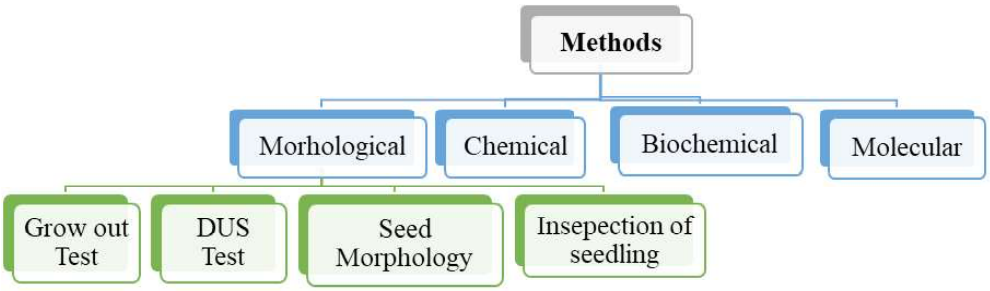

Figure 1: Flowchart on Methods of Varietal identification

Morphological Methods

It involves the examination of physical traits such as seed size, shape, colour, and texture. These methods are often the first step in identifying seed varieties and are widely used due to their simplicity and cost-effectiveness.

Grow-Out Test

Grow-out test (GOT) is a crucial technique for varietal identification in agriculture. It involves cultivating a sample of seeds in controlled conditions to observe the morphological

characteristics of the resulting plants and verify that they correspond to the expected traits of the specified variety. The GOT is the official method used to monitor the seed lot's genetic purity. It prevents genetic contamination by acting as both a "post-control" and a "pre-control" test. It is a requirement for seed certification of hybrids of some species, including cotton, castor, musk melon, and brinjal, according to official Indian rules (Kavimandan & Khan, 2011).

According to the Seeds Act of 1966, the test must be performed to verify the seller's label regarding the genetic purity status of the seed lot. The GOT is a tool that can be used to assess the effectiveness of the inspector or the certifying body.

Procedure of GOT

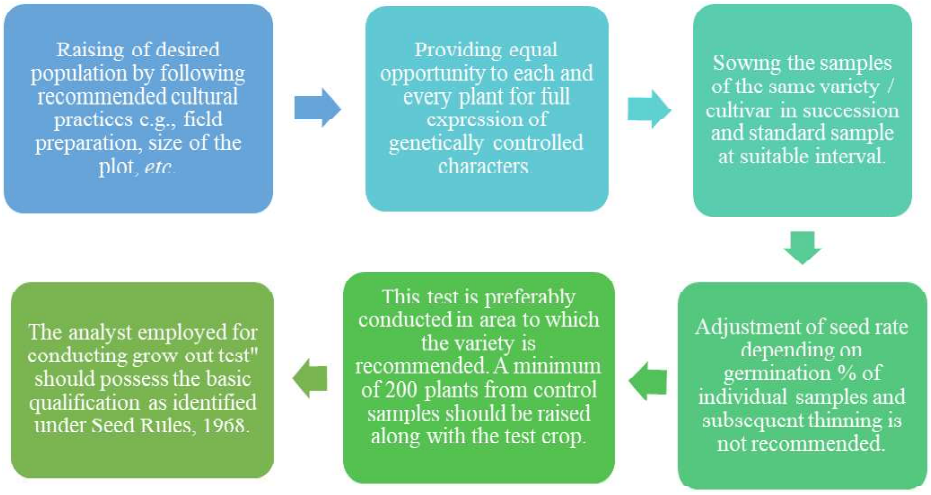

Figure 2: Procedure of Grow out test (Kumar *et al.*, 2021)

Results Reporting

Results reporting for grow-out tests involves several key components. Firstly, it is imperative to record the percentage of various species, cultivars, or off-type plants identified in the findings. If the sample received differs from the claimed cultivar, this variation must be accurately documented in the report. Additionally, should the presence of plants from other cultivars surpass 15%, the report must specify that the sample comprises a blend of distinct cultivars. Conversely, if the grow-out test reveals no significant deviations or discrepancies from the provided cultivar or species name, the report should indicate that no noteworthy observations were made.

DUS Test

DUS stands as Distinctness, Uniformity & Stability. It is a standardized method used for the identification and registration of plant varieties. This test is crucial for ensuring that new

plant varieties are distinct, uniform, and stable, which are key criteria for granting plant breeders' rights (Gilliland and Gensollen, 2010).

For seed varietal identification, certain criteria must be met to ensure distinctness, uniformity, and stability (DUS). Distinctness refers to the clear differentiation of a variety from any others, particularly regarding at least one identifiable characteristic. Uniformity is essential for the variety to be consistently described and characterized, indicating a level of homogeneity within its traits. Stability is crucial as it denotes the ability of the variety to maintain its essential characteristics over successive generations, even under varying environmental conditions and propagation methods. These criteria collectively serve as fundamental benchmarks in the assessment and classification of plant varieties, facilitating their recognition and ensuring reliability in agricultural practices and breeding programs.

A DUS test is typically carried out over the course of two consecutive growth seasons in a glasshouse in the field. Many mostly morphological traits are noted during this time on the new (or candidate) variety as well as on related varieties in what is referred to as "Common Knowledge." If there are differences, they are measured and observed according to internationally recognized protocols. In compliance with the rules and specifications set forth by the International Union for the Protection of New Varieties of Plants (UPOV), it is carried out both in a laboratory and on a trial field. Common knowledge holds that a variety that has been registered and awarded rights is a registered variety.

Methodology

The DUS test involves several crucial steps

a. **Selection of Characteristics**: The test targets specific morphological and physiological traits that are relevant and quantifiable. These traits are predetermined and standardized for each crop species.

b. **Growing Trials**: Samples of the candidate variety and comparator varieties are cultivated in controlled field trials. These trials span at least two growing seasons to ensure consistent results.

c. **Observation and Recording**: Detailed observations and measurements of the selected traits are conducted at various stages of plant growth. Traits assessed may include plant height, leaf shape, flower colour, and fruit size.

d. **Data Analysis:** The gathered data is analysed to ascertain whether the candidate variety meets the criteria for distinctness, uniformity, and stability. Statistical methods may be employed to ensure precision and objectivity.

Seed Morphology Test

Their examination involves the use of appropriate and necessary magnification to analyse morphological features. The size and form of the grain, the base of the lemma, the hairs in the vertical crease, the hairs in the rachilla, the deviation of the lateral dorsal nerve, *etc*. Full

daylight or light with a narrow spectrum, such as UV light, is used to study the colour properties. In certain species, the inner structure of the seed coat and its variations are studied using electron microscope scanning.

Examination of Seedlings

On seedlings, numerous useful varietal identification tests have been carried out, providing several noteworthy benefits. Compared to observations of ungerminated seeds, seedling testing can yield more detailed information since the early growth stages can display unique morphological and physiological traits that are essential for precise identification. Furthermore, these tests take a lot less time than field grow-out tests, which need a whole growing season in order to examine mature plants. Shorter turnaround times for seedling test results enable speedier decision-making in breeding programs and quality assurance. Reliability is increased by conducting these studies in controlled settings, such as greenhouses, which lessen external unpredictability. All things considered, seedling tests are useful and effective, aiding in the creation and upkeep of premium, pure seed kinds.

Chemical Method

Additional benefits for varietal identification come from a variety of chemical or laboratory testing. These chemical tests may be carried out under controlled circumstances at any time of year and are very rapid and simple to perform. The seeds' colours differ as shown by the chemical testing. Additional advantages for achieving more authentic results include the study of phenotypic characteristics in conjunction with chemical and biochemical approaches. The chemical agents in these assays interact with the seed to aid in varietal identification (Vijayalakshmi & Vijay, 2009). Some of the major chemical methods are:

(i) **Phenol Test**: Walls (1965) recommended using the Standardized Phenol Test for varietal purity testing, and this was done. In terms of variations, this test is very specific. A monogenically regulated response is a phenol reaction. The oxidation of externally provided phenol into quinones is carried out by the enzyme polyphenol oxidase (PPO) or tyrosinase, which is found in the seed coat. Quinones' subsequent polymerization produces pigments like melanin, which is what gives seeds their brown colour. The coloured reaction, which is rated on a scale of 0 to 9, where 0 represents a negative reaction or no change, is used as a basis for classifying the strength of the colour received. Gradually, the colour intensifies from light brown to deep black. It is mostly applied to mustard, foxtail millet, sorghum, wheat, and rye but also has been standardized for rice by Kumar *et al.*, (2023).

(ii) **Potassium Hydroxide (KOH) Test**: Three replications of 100 seeds each were steeped for two hours at room temperature in a 5% KOH solution. After an hour, seeds start to change colour. The genotypes of the seed were divided into three groups, namely light brown, reddish brown, and dark brown, according to the intensity of the colour. The

length of the test varies depending on the seeds used and their seed coat. Light and dark pigmented testa are looked for during the test.

(**iii**) **Sodium Hydroxide (NaOH) Test:** Three replications of 100 seeds each are steeped for one hour at room temperature in a 5% NaOH solution. After an hour, seeds start to change colour. The genotypes are categorized according to the seed's level of colour intensity. It is applied to several cereal crops.

(**iv**) **Peroxidase Test**: For the purpose of this test, 60 seeds are soaked in water for 24 hours, and then 15 seeds are incubated for 20 minutes in 2.5 ml of guaiacol solution (0.05%). After removing 2 ml of guaiacol, 0.2 ml of H_2O_2 (0.1%) was added. At 480 nm, it was measured using a DU 64 spectrophotometer. After adding H2O2, the enzyme activity was calculated every minute for 5 minutes. Depending on the colour intensities of the seedling, the outcome is classified (Mandal *et al.*, 2001).

(v) **2,4 D Auxin test:** It is examined how the 2,4-D test affects seedlings at a concentration of 5 ppm. Following a 7-day period, the seedlings are assessed. Ten seedlings are chosen at random, and their length, which includes both shoot and root length, is measured in centimetres. Each crop has a different technique and set of findings. Inferences are made based on the variations attained.

(**vi**) Top of Form

(**vii**) Bottom of Form

(**viii**) **Gibberellins soaking test:** Seedlings are grown on germination paper towels that have been wet with a 100 ppm GA solution. After observing the growth of the seedlings for seven to ten days, cultivars are categorized as high or low based on how they respond to the elongation of the roots and shoots.

(**ix**) **Alkaloid test:** The presence or lack of an alkaloid is a distinguishing characteristic for *Lupinus species*. Water is used to soak seeds for a whole day. Cut thin slices of seeds and arrange them over a white surface on a glass platter. One or two drops of Lugol's solution are then added. The presence of alkaloid is indicated by the formation of a characteristic brownish red precipitate.

(**x**) **Iodine test:** Its primary purpose is to distinguish weed seeds from millet seeds. Weed seeds turn brown after soaking in a 5% iodine solution for five to seven minutes, but millet seeds stay the same or turn pale green.

Biochemical Methods

Biochemical markers, encompassing the chemical fingerprints and internal processes of living organisms, offer a pivotal avenue for distinguishing between different varieties. These markers, including proteins, isozymes, enzymes, and carbohydrates, manifest in diverse forms across species, allowing for the identification of specific varieties based on the presence or absence

of particular biochemical components. Various analytical methods are employed for detecting these biochemicals, with electrophoretic analysis emerging as a prominent and cost-effective technique for assessing cultivar identity and purity in seed batches. Techniques like Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Acid Polyacrylamide Gel Electrophoresis (A-PAGE) enable swift and dependable analysis of seed proteins and isozymes, providing critical insights into the genetic composition of plant cultivars and their parental lines, particularly within the context of hybrid seed production.

SDS-PAGE and A-PAGE stand out as two of the most accessible and widely utilized biochemical methods for cultivar identification, offering practicality and reliability in the assessment process. SDS-PAGE technology, in particular, holds promise for its ability to furnish consistent profiles of seed storage proteins, which remain relatively stable amidst environmental fluctuations. This stability not only ensures the accuracy of cultivar assessments but also contributes to the economic evaluation of plant varieties (Rao et al., 2012).

Molecular Methods

Electrophoresis

This is the most recent cultivar identification technique, based on isoenzyme activity, protein banding, and DNA banding. Here, single seeds are extracted for protein and esterase's after being defatted. Electrophoresis on polyacrylamide gel is used to separate the extracted proteins or esterase's (Wang *et al*., 1994). The variants can be distinguished and recognized based on the protein and enzyme banding patterns. Using an agarose gel electrophoresis, DNA is separated.

Principle

The movement of a charged particle under the influence of an electric field is referred to as "electrophoresis." Proteins are extracted from seeds by grinding them. After that, the isolated proteins are put into gel matrix wells (usually made of agarose or polyacrylamide). Proteases move through the gel in response to an electric current. The size and charge of the proteins affect the rate of migration.

Protein Profiling of Seeds: Different seed varieties produce unique protein profiles. By comparing the protein banding patterns from electrophoresis against known standards, specific seed varieties can be identified. This method is highly effective for distinguishing between closely related varieties.

Polymerase Chain Reaction (PCR)

America's Nobel Laureate biochemist Kary Mullis created PCR for the first time in 1983. Making several copies of a specific DNA region is possible in a lab setting using the PCR technology. Through multiple cycles, PCR combines the principles of nucleic acid replication and complementary nucleic acid hybridization. It causes a factor of 10^7 exponential creation

of the targeted DNA/RNA sequences in a comparatively short amount of time. Primer for PCR and *Taq* polymerase are the two fundamental ingredients in PCR. Originating from *Thermus aquaticus*, *Taq* polymerase is the DNA polymerase commonly utilized in PCR. Because of its exceptional thermal stability, *Taq* DNA polymerase is the perfect choice for PCR.

Principle: Extraction and Analysis of DNA: Chemical techniques are used to break down the cell walls and membranes of seeds in order to extract the genetic material (Tanksley & Jones, 1981). While RFLP (Restriction Fragment Length Polymorphism) examines the patterns of DNA fragments created by restriction enzyme digestion, PCR techniques are used to amplify particular DNA sections.

Applications

Genetic Markers: Different seed kinds are linked to certain DNA sequences, or markers. It is possible to acquire reliable identification by comparing these markers with those of known kinds. This technique is very accurate and can tell apart even closely related types.

Biochemical Markers

These are heritable DNA sequences that may be discovered using methods like Southern hybridization or PCR. They are also phenotypically neutral, developmentally and environmentally stable. Over the past ten years, molecular techniques have been used to identify plant cultivars by creating molecular markers that identify variations in DNA sequences between cultivars. For each cultivar, highly specific marker profiles also referred to as DNA fingerprinting can be created and utilized to identify the cultivar.

Principle: 1) Extraction of Biochemical Compounds

(i) Sample Preparation: To enhance the extraction surface area, seeds are finely powdered. Then, different solvents (such as water, ethanol, or methanol) are added to this powder in order to extract different metabolites, including lipids, proteins, enzymes, and amino acids.

(**ii) Filtration and Centrifugation:** To separate soluble components from insoluble debris, the extract is centrifuged after being filtered to remove solid particles.

(2) Chromatography and Spectroscopy: **High-Performance Liquid Chromatography (HPLC)**: **Principle**: Based on how a molecule interacts with the stationary phase (column) and the mobile phase (solvent), HPL separates it. Retention times are the intervals at which certain chemicals elute (leave the column).

Procedure: The seed extract is injected into the HPLC system. The mobile phase carries the sample through the column, and the separated compounds are detected using a UV detector, a fluorescence detector, or a mass spectrometer.

Analysis: Each compound produces a peak at a specific retention time. By comparing these retention times and peak areas with those of known standards, the presence and concentration of various metabolites can be determined.

(3) Mass Spectrometry (MS): **Principle**: Compounds are identified and quantified using mass-to-charge ratios (m/z) in mass spectrometry. It frequently comes after HPLC to offer comprehensive structural details about the metabolites (De Hoffmann & Stroobant, 2007).

Procedure: The separated compounds from HPLC are ionized and fragmented in the mass spectrometer. These ions are then detected and plotted as a spectrum, showing the m/z values.

Analysis: The resulting mass spectrum is compared against known databases to identify the compounds. The intensity of the peaks indicates the quantity of each metabolite.

Why is Seed Varietal Identification Important?

Seed varietal identification is crucial for several reasons, including ensuring genetic purity, supporting breeding programs, complying with legal requirements, and building market confidence (Dongre *et al*., 2011). Confirming the genetic purity of seeds ensures that the sown seeds remain true to type, preserving the unique characteristics of each variety and preventing genetic contamination. Accurate identification aids breeding programs by helping in the selection of parent plants, which is essential for developing improved and disease-resistant crops. Furthermore, many countries have regulations and certification processes that mandate the accurate identification of seed varieties to protect farmers and consumers from fraudulent practices. Ensuring the authenticity of seed varieties also builds trust among farmers, seed companies, and consumers, thereby promoting the use of high-quality seeds for better crop yields. Varietal identification distinguishes between different varieties within a single crop species, addressing variable production and yield outcomes. This process allows farmers to select varieties that best suit their soil and available resources, enhancing their productivity and economic status. Indirectly, this contributes to the national economy by providing farmers the opportunity to choose high-performing varieties with resistance to insects, pests, and diseases (Azizi *et al*., 2021).

Conclusion

In contemporary agriculture, methods for identifying seed varietals are crucial for guaranteeing the genetic integrity, legitimacy, and excellence of seed supply. Various techniques, such as morphological, chemical, biochemical, and molecular studies, are essential for improving crop quality and yield. Reliable and affordable methods for identifying seed varieties include PCR and chromatography, as well as sophisticated techniques like SDS-PAGE, A-PAGE, and electrophoretic analysis. Precise identification promotes market confidence, regulatory compliance, and selective breeding—all of which are crucial for sustainable agricultural practices and the general increase in agricultural productivity.

References

Anis, G., Hassan, H., Sherif, A. E., Saneoka, H., & Sabagh, A. E. (2019). Evaluation of new promising rice hybrid and its parental lines for floral, agronomic traits and genetic purity assessment. *Pakistan Journal of Agricultural Sciences*, *56*(3), 567-576.

Azizi, M. M. F., Lau, H. Y., & Abu-Bakar, N. (2021). Integration of advanced technologies for plant variety and cultivar identification. *Journal of biosciences*, *46*, 1-20.

Cooke, R. J. (2020). Variety identification: modern techniques and applications. In *Seed Quality* (pp. 279-318). CRC Press.

De Hoffmann, E., & Stroobant, V. (2007). Mass spectrometry: principles and applications. John Wiley & Sons.

Dongre, A. B., Raut, M. P., Bhandarkar, M. R., & Meshram, K. J. (2011). Identification and genetic purity testing of cotton F1 hybrid using molecular markers. *Indian Journal of Biotechnology*, *10*, 301-306.

Gilliland, T. J., & Gensollen, V. (2010). Review of the protocols used for assessment of DUS and VCU in Europe–Perspectives. In *Sustainable use of genetic diversity in forage and turf breeding* (pp. 261-275). Springer Netherlands.

Kavimandan, B., & Khan, Z. H. (2011). Sorghum cultivar identification by seed protein profile-Better option for Grow Out Test (GOT). *Bioscience Discovery*, 2(3), 367-377.

Korir, N. K., Han, J., Shangguan, L., Wang, C., Kayesh, E., Zhang, Y., & Fang, J. (2013). Plant variety and cultivar identification: advances and prospects. *Critical reviews in biotechnology*, *33*(2), 111-125.

Kumar, S. J., Susmita, C., Agarwal, D. K., Pal, G., Rai, A. K., & Simal-Gandara, J. (2021). Assessment of genetic purity in rice using polymorphic SSR markers and its economic analysis with grow-out-test. *Food Analytical Methods*, *14*, 856-864.

Kumar, S., Chakrabarty, S. K., & Lal, S. K. (2023). Standardization of phenol colour reaction test in rice (Oryza sativa) varieties. *Plant Physiology Reports*, *28*(2), 345-348.

MacLachlan, I. R., McDonald, T. K., Lind, B. M., Rieseberg, L. H., Yeaman, S., & Aitken, S. N. (2021). Genome-wide shifts in climate-related variation underpin responses to selective breeding in a widespread conifer. *Proceedings of the National Academy of Sciences*, *118*(10), e2016900118.genetic

Mandal, A. B., Maiti, A., Chowdhury, B., & Elanchezhian, R. (2001). Isoenzyme markers in varietal identification of banana. *In Vitro Cellular & Developmental Biology-Plant*, *37*, 599-604.

Mueller, N. G., & Flachs, A. (2022). Domestication, crop breeding, and genetic modification are fundamentally different processes: implications for seed sovereignty and agrobiodiversity. *Agriculture and Human Values*, *39*(1), 455-472.

Rai, B., Gupta, S. K., & Pratap, A. (2007). Breeding methods. *Advances in botanical research*, *45*, 21-48.

Rao, P. S., Bharathi, M., Reddy, K. B., Keshavulu, K., RAO, L. S., & Neeraja, C. N. (2012). Varietal identification in rice (Oryza sativa) through chemical tests and gel electrophoresis of soluble seed proteins. *The Indian Journal of Agricultural Sciences*, *82*(4), 304-11.

Tanksley, S. D., & Jones, R. A. (1981). Application of Alcohol Dehydrogenase Allozymes in Testing the Genetic Purity of F1 Hybrids of Tomato1. *HortScience, 16*(2), 179-181.

Vijayalakshmi, B., & Vijay, D. (2009). Development of seed keys for varietal identification in Rice (Oryza sativa L.) using chemical tests. *Seed Research*, *37*(1&2), 56-61.

Walls, W.E. (1965). A standardized phenol method for testing seed for varietal purity Handbook on Seed Testing. Association of Official Seed Analysts (28) pp. 7.

Wang, C., Bian, K., Zhang, H. X., Zhou, Z. M., & Wang, J. A. (1994). Polyacrylamide gel electrophoresis of salt-soluble proteins for maize variety identification and genetic purity assessment. *Seed Science and Technology*, 22(1), 51-57.