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Quality Assurance Techniques

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QUALITY ASSURANCE TECHNIQUES

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Emphasis: Variety Determinations

HYPOCOTYL COLOR TEST

The hypocotyl color test may be used as an aid for the varietal identification of soybean seedlings. Some varieties of soybeans produce white flowers while other varieties produce purple flowers. Observing the hypocotyls of 3 to 4 inch seedlings when grown under high intensity light can eliminate the necessity of growing plants until they flower to observe or verify flower color. Seedlings of white flowered varieties have green hypocotyls, while those of purpled flowered varieties have dark or purple hypocotyls.

Materials Needed

1. A greenhouse, window box, lighted walk-in germinator, or other facility where the temperature can be maintained at 75-86F.

2. Greenhouse benches, soil trays or other containers which can be used for planting.

3. Sand or a sand/soil mix for use as the substrate.

4. Good fluorescent lighting should be available as the seedlings being to emerge when natural light is not available, i.e., greenhouse.

Procedures

1. Plant two or more replicates of 100 seeds each in a greenhouse bench or soil tray and if not in greenhouse place in a window box, walk-in germinator, etc.

2. Turn on lights as seedlings begin to emerge if natural lights not available.

3. Observe seedlings for hypocotyl color when they are 3-4 in. tall. Seedlings that have green hypocotyls will produce white flowers while those that have purple or dark hypocotyls will bear purple flowers.

Conclusion

The use of the hypocotyl color test is one of several procedures which can be used in the laboratory or greenhouse to help in distinguishing among soybean varieties.

Note

The hypocotyl (or seedling) color test may be applicable to other kinds of seed, e.g., sorghum, wheat.

THE PEROXIDASE TEST

The seed coat of some soybean varieties contains a "high activity" peroxidase enzyme. Other varieties have seed coats which have a form of peroxidase with "low activity". This difference in peroxidase enzyme activity may be used to distinguish between some varieties of soybeans.

Materials Needed

1. A 0.1% aqueous solution of hydrogen peroxide (H_2O_2) is used. Ordinarily, a 3% hydrogen peroxide solution can be purchased from a drug store. To prepare a 0.1% aqueous solution, 1 ml (or cc) of 3% hydrogen peroxide should be added to 29 ml (or cc) of water. The 0.1% solution should be stored in a refrigerator when it is not being used.

2. A 0.5% guaiacol solution is also needed. Guaiacol can be obtained from a chemical supply company (see below). To obtain a 0.5% guaiacol solution, 0.5 ml (or cc) of guaiacol should be added to 100 ml (or cc) of water and stirred until mixed. This solution should also be stored in a closed container in a refrigerator when it is not in use.

3. Test tubes will be needed in which to place the seed coats, guaiacol and hydrogen peroxide solutions. These can be obtained from a scientific supply company (see below).

Procedures

1. Remove the seed coat from individual soybean seed and place in separate test tubes (i.e., one seed coat per test tube). The seed coats can be removed from dry seeds or if it is difficult to remove the seed coat, the seed may be placed in boiling water for three minutes to loosen the coat. It is important to remove all fragments of the cotyledons before placing the seed coat in test tube.

2. Add 10 drops of the 0.5% guaiacol in each test tube and wait 10 minutes.

3. After 10 minutes, add 1 drop of 0.1% hydrogen peroxide solution to each test tube.

4. Record the results after five minutes. If the solution turns a dark redish brown the reaction is considered positive (+). If the solution remains clear the reaction is considered negative (-). *

Conclusion

The peroxidase test is another method that may help differentiate between seeds of different soybean varieties. Some varieties give a positive (+) reaction for peroxidase while others give a negative (-) reaction.

Sources of Supplies

1. Guaiacol, chemical reagent. Fisher Scientific, 711 Forbes Ave., Pittsburg, PA 15219.

2. Test Tubes, general purpose, Pyrex, 15 x 125 mm. Fisher Scientific (see above).
   Note: There are other sources of supplies needed. Fisher is only one.

*If a sample gives both positive and negative results on the seed coats tested, the seed coats of 25 seeds should be tested.

THE POTASSIUM HYDROXIDE TEST FOR RED RICE (*Oryza sativa* var.)

Red Rice (*Oryza sativa* var.) is a serious noxious weed in all rice producing areas. Because of variation in time of emergence and maturity some of the red rice contaminants in cultivated rice will be somewhat immature and the red color of the pericarp will not be fully developed. Potassium hydroxide (KOH) can be used to detect and/or verify red rice caryopses in samples of cultivated rice varieties.

Materials Needed

1. A 2% aqueous KOH solution is needed. KOH can be obtained from a chemical supply house (see below). The solution may be prepared by dissolving 2 grams of KOH pellets in 100 cc of water, or by appropriate dilution of an off-the-shelf solution.

2. A small dish, petri dish or test tube may be used in which to place questionable caryopses.

3. An eye dropper is helpful to dispense the KOH solution.

Procedures

1. Suspect red rice seeds should be hulled by hand or machine and examined carefully under magnification. Fully mature seed (caryopses) with definite red pericarp are red rice and need not be tested further. A seed with a pericarp of questionable or indefinite color (because of immaturity, diseases, etc) should be subjected to the KOH test.

2. Place the hulled questionable seed in small glass dishes and "drop on" 2-3 drops of the 2% KOH solution. A white porcelian plate with seed spaced 1½ inches apart makes an excellent background for the test.

3. Observe seeds "immersed" in the drops of KOH. Usually the KOH solution will turn red or dark orange within a few minutes if the seed is a red rice. If the seed is not a red rice, the KOH remains colorless or might turn a faint yellow. Although the reaction is usually complete in 10 minutes, observations should be continued for about 20 minutes to "make sure".

Conclusion

A 2% aqueous KOH solution can be used to verify whether a questionable caryopsis is a red rice or a cultivated (white) rice. A caryopsis of red rice will turn the KOH solution a red or dark orange color in 3 to 20 minutes. The KOH around the caryopsis of cultivated (white) rice remains colorless or faint golden yellow.

Source of Supply

KOH can be purchased as pellets or as a prepared solution (say 45%). Two grams of pellets in 100 cc water will give the desired 2% KOH solution. In the case of the 45% off-the-shelf solution, add one part of the solution to 21 parts water to obtain an approximate 2% solution.

Potassium Hydroxide (KOH)pellets, or Potassium Hydroxide 45% solution: one source is Fisher Scientific, 711 Forbes Ave., Pittsburg, PA 15219.

References

1. Rosta, K. 1975. Variety determination in rice (Oryza sativa L.). Seed Sci. and Tech. 3(1):161-169.

2. Staff, Louisiana State Seed Testing Laboratory. 1980. A potassium hydroyide test for the confirmation of red rice (Oryza sativa var.) seed. Assoc. Off. Seed Analysts NewsLetter 54(1):68-69.

THE PHENOL TEST

The phenol test is used as an aid in the identification of wheat (Triticum aestivum), Kentucky bluegrass (Poa pratensis), Oats (Avena sativa), barley (Hordeum vulgare) and rye (Secale cereale) varieties.

The phenol test is based on a phenol oxidase activity. The phenols are oxidized by the enzymes present in the pericarp, aleurone and other seed structures. As a result of enzymatic oxidation activities, dark colored, insoluble pigments (melanins) are formed. The amounts and kinds of the various phenol oxidase type enzymes present in the seed cause varying degrees of coloration in the pericarp.

Materials Needed:

1. Phenol is carbolic acid in the form of loose crystals. Carbolic acid crystals may be obtained from a chemical supply company, or, alternatively, an 89-90% solution of phenol can be purchased.

2. Distilled water is needed to prepare the solution.

3. Large (15 cm) plastic petri dishes or other containers of a similar size are needed. Good quality white filter paper of size suitable for containers used is also needed.

4. A small metal container is needed in which to heat the carbolic acid crystals over a stove if the crystals are used.

5. Graduated cylinders or other measuring vessels are needed to measure the melted phenol and water.

6. A flask should be available in which to store the excess phenol solution.

Procedure:

1. Prepare a 1% solution of phenol. This is done by first heating and melting the carbolic acid crystals until they are in liquid form. To prepare a 1% solution five ml or cc of melted carbolic acid are added to 495 ml (or cc) of distilled water. Alternatively, a 1% solution of phenol can be prepared by diluting a liquid preparation (90%): 1.1 cc of shelf solution in 98.9 cc water.

2. Soak 200 seeds in distilled water for 16 hours. Drain and place the seeds in two 15 cm plastic petri dishes containing two layers of white filter paper per dish soaked in 1% phenol solution. Place seeds in rows of ten seeds to make 100 per dish (if the sample is wheat, they are placed crease side down).

3. After 1 1/2 to 2 hours read the percentage of seeds with the varying degrees of coloration. The seeds will continue to darken as they remain in the solution. However, color differences are more difficult to see after 2 hours of staining time.

Conclusion:

Different varieties of wheat exhibit different degrees of staining when exposed to phenol solution for 1 1/2 to 2 hours. To draw a conclusion whether a particular lot of wheat is pure, one must know the phenol reaction of the particular variety. If foundation seed of the variety is available one can compare the phenol reaction of the foundation seed with the seed in question.

Notes:

Phenol should be used with caution since it is toxic. The fumes from phenol should not be inhaled; therefore, it is best if fumes can be drawn away from a technician with an exhaust fan. A technician working with phenol should avoid rubbing the solution in his eyes.

Extra phenol may be stored in a refrigerator for three or more weeks.

Source of Phenol (Carbolic Acid) and Filter Paper:

Carbolic acid and filter paper may be obtained from most chemical supply houses. One source is listed here for your information:

Fisher Scientific
711 Forbes Avenue
Pittsburg, PA 15219