Specific Isozyme Pattern of Rice Seed cv. Kaodawkmali 105 and cv. Chainat 1.

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Abstract

Genetic purity of rice seed is one of the factor for good quality rice seed production. Using mixed rice seed have been practiced recently by Thai seed farmers, since the mixed seed used are bought from local traders. Mixed seed from rice cv. Kaodawkmali 105 (KDML105) which is more expensive with cv. Chinat 1 (CN1) is most practiced. The number of this mixed seed used is heavily increased because of the trader try to lower the seed price and their morphological appearances are closed. Therefore, the international rice trading agencies in Thailand has brought up this problem into public discussion more often. This experiment purposed to find the better cheaper way from well known technique e.g. DNA fingerprint to simple specific isozyme electrophoresis in order to develop quicker method to solve the problem from expensive laboratory analysis. The determination for the specific isozyme patterns of rice seed cv. KDML105 and cv. CN1 were done by using method of polyacrylamide gel electrophoresis technique (PAGE). Five enzymes: esterase (EST), glutamate oxaloacetate trasaminase (GOT), leucine amino peptidase (LAP), malic enzyme (ME) and malate dehydrogenase (MDH) were assayed. It was found that the esterase enzyme showed distinguishly significant difference. The isozyme pattern from rice seeds cv. KDML105 and cv. CN1 is differently noticed. No distingui shly differences of the isozyme pattern between rice seeds cv. KDML105 and cv. CN1 in other enzymatic patterns. Thus, esterase enzyme electrophoresis technique could be used to separate the rice seed mixture of cv. KDML105 and cv. CN1. Further research is recommended.

2 Background and Aim of the Study

Genetic purity means the absence of other varieties seeds or the same crop species as well as of other crop species. Genetic purity ensures that the seed is of the variety under certification, and that there is no mixture from other varieties or other crops. The standard of genetic purity is very high, the amount of mixture permitted ranges from 0-0.1 per cent. In most of the cases, mixture by seeds of other crop species is permitted to a small degree (up to 0.1 per cent), but mixture by seeds of other varieties of the same crop is generally not permitted. In a rice seed trading, one of the challenges is the production and supply of adequate quantities of pure seed to the farmers. Maintenance of high level of genetic purity of seed is essential to exploit the seed observed in this crop trading. The certified seed is annually produced by progressive farmers according to standard seed production practices. To be certified, the seed must meet the prescribed requirements regarding purity and quality. Purity of rice seed lots is assayed conventionally by verification on a sample of the seed that is to be marketed. The verification involves growing plants to maturity, assessing several morphological, floral characteristics and molecular analysis that distinguish the variety of seed.

Molecular marker has been widely used to verify in seed varieties purity test. The majority of the work utilizing molecular marker in seed production has to base on genetic mapping using various DNA marker systems. In rice, molecular marker techniques are abundant and well distributed throughout the genome. For rice seed production, a molecular marker using procedure for detecting purity has been standardized and uses rice (Oryza sativa L.) seedlings, which could be used for detection of mixture in seed lots. The extent of mixed seed within parental lines of rice seed was assessed and the results suggest that a appropriately chosen molecular marker should be sufficient for assessing seed purity.
Modern methods for varietals verification as electrophoresis of seed storage proteins and testing of seed storage proteins with PAGE, SDS and Isozymes were applied with wide range of crops e.g. *Zea mays*, *Triticum aestivum*, *Cucumis sativa*, and *Oryza sativa*. Polyacrylamide gel electrophoresis is a technique which separates isozyme of varying rice seed characteristic. When rice seed isozymes are separated the resulting banding pattern is used as a fingerprint for that particular variety. Using this technique for varietal identification is fast and economical. Primary use of polyacrylamide gel electrophoreses tests has been used in variety verification by Crop Improvement Associations, the use is growing among seed companies and farmers in variety verification of purchased seed. The aim of the study was to determine specific isozymes of rice seed cv. KDML105 and cv. CN1.

3 Methods
3.1 Plant material
Rice seed material was soaked in water for 2 days or till radicle emergence. Seedling growth were then taken place in the basket under optimum light and water. They grew under room temperature, rainfall protection, 2 times. The 15 days old rice seedling from each plant was used for enzyme extraction and solution samples for isozyme analysis. Five enzymes: esterase (EST), glutamate oxaloacetate trasaminase (GOT), leucine amino peptidase (LAP), malic enzyme (ME) and malate dehydrogenase (MDH) were determined.

3.2 Electrophoresis
Rice seedling was milled with liquid nitrogen and extracted by extract buffer 1 ml. for 0.5 g. of plant sample (Pooler and Simon, 1993). Samples were centrifuged at 14,000 rpm at 4 °C for 20 min., the extracted sample tubes were kept in -20°C refrigerator for electrophoresis process. Preparing separating gel 10% by using Acrylamide-bisacrylamide 0.62 ml (the ratio of acrylamide to bisacrylamide was 30:0.8), Tris-HCL 3 M pH 6.8 1.25 ml and Water 4.81 ml, APS 1.5% 0.5 g and TEMED 10 µl. Gases were removed by pumping. Preparing stacking gel 60 % by used Acrylamide-bisacrylamide 3.33 ml, Tris-HCL 3 M pH 8.8 1.25 ml, Water 1.8 ml, APS 1.5% 0.25 g and TEMED 15 µl then cleaned separating gel by deionized water and Gases were removed also by pumping. Put on the electric supply 15.0 mA for stacking gel and 25 mA for separating gel (150-200 V). The completed gel was determined for the appeared isozyme patterns by staining the enzymes. Put the gel on the plate and poured the staining solution on it and then incubated the gel for 15-60 min. at 37 °C until the isozyme pattern appeared (Vellejoe, 1983). Isozyme pattern assessment were done by counting the appeared and disappeared isozyme pattern and 1 was used for appeared isozyme pattern and 0 was used for disappeared isozyme pattern (Sneath and Sokal, 1973).

4 Result and Discussion
The patterns of EST appeared in red-purple colors and the location of this pattern was appeared on top of acrylamide slap gel and Rf band values appeared on 4 positions which were 0.69 , 0.79, 0.85 and 0.96 respectively (A). EST patterns can be used to separate the difference between rice seed cv. KDML105 and cv. CN1. at the second position of patterns found EST pattern in rice seed cv. KDML105 but could not be find EST pattern in CN1. The EST was reported as the study on seed germinating of *Brassica sp.* (Nakai, 1970).

The patterns of GOT appeared in green-dark blue colors and the location of this pattern was appeared on top of acrylamide slap gel and Rf band values appeared on 2 positions which were 0.79 and 0.85 (B). The patterns of LAP appeared dark blue colors and the location of this pattern was appeared on top of acrylamide slap gel and Rf band values appeared on 2 positions which were 0.81 and 0.91 (C). The patterns of MDH appeared dark blue colors and the location of this pattern was appeared on top of acrylamide slap gel and Rf band values appeared on 2 positions which were 0.74 and 0.81 (D). The patterns of ME appeared purple-dark blue colors and the location of this pattern was appeared on top of acrylamide slap gel and Rf band values appeared on 6 positions which were 0.51, 0.62, 0.67, 0.75, 0.78 and 0.89 respectively (E). The results from this experiment corespondence with the report from Maria , et al (2002) which found also distinguishly genetic differentiation patterns of *Cerastium arvense* (Caryophyllaceae). The isozyme patterns between rice seed cv. KDML105 and cv. CN1 can not be used from the patterns of GOT, LAP, MDH and ME. The appeared and disappeared isozyme patterns from GOT, LAP, MDH and ME can not be used to distinguish the differentiation between rice seed cv. KDML105 and cv. CN1. Since they looked exactly the same in both varieties. Thus application
Electrophoresis technique from this experiment can be used to verify genetic purity of rice seed cv. KDML105 and cv. CN1.

5 Conclusion
Appeared and disappeared EST pattern analysis can be used to separated the differentiation between rice seed cv. KDML105 and cv. CN1. by polyacrylamide gel electrophoresis technique.

Reference


