Effective Methods for Dormancy Breaking of 15 New-Improved Rice Varieties to Enhance the Validity of Germination Test

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ABSTRACT

Dormancy is acharacteristic of seeds, which is genetically inherited and is an important factor in the germination of seeds. Dormancy in rice serves as a mechanism of survival by protecting the seed from germinating in the mother-plants, however it becomes a problem in germination evaluation as part of seed quality testing when methods for overcoming dormancy is not effective.Seven different treatments were used to determine seed dormancy of 15 new improved-varieties. Results showed all varieties tested have a diversity of dormancies. A total of 15 varieties showed intensity of dormancy varied between 47-98%.Persistency of dormancy of the 15 varieties ranged between 2-8 weeks. At the beginning of seed storage (0 week), the results showed that 14 out of 15 varieties treated by drying at temperature of 50^{o}C for 48 h and continued by soaking the seeds in 10 ppm GA_3 solution for 24 h gave means value of seed germination ≥85%, whereas the control seeds of 15 tested varieties showed means value of germination below 60%. This method of breaking seed dormancy is considered to be effective and available to be used by seed analysts on rice seed germination testing specifically for those 14 varieties tested.

Key words: Germination, breaking dormancy, improved-variety, rice

INTRODUCTION

Dormancy is an internal condition of the seed that impedes its germination under otherwise adequate hydric, thermal, and gaseous conditions (Benech-Arnold et al., 2000).Baskin and Baskin (2001, 2004) stated that seed dormancy can be caused by the presence of germination inhibitors, impermeable seed coats, or underdeveloped embryo.Plant growth regulators such as GA (giberellic acid) and IAA (indoleatic acid) (HilhorstKarssen, 1992; Iglesias and Babiano, 1997); chemicals such as KNO_3 (potassium nitrate) (Hartmann et al., 1997) and hot water treatments (Hermansen et al., 1999) have been recommended to break dormancy and enhance germination. In dormant-seed, dormancy is an important trait, because it provides a strategy for seeds to spread germination in time in order to reduce the risk of premature death in an unfavourable environment or to prevent pre-harvest sprouting in humid areas.

Variation in seed dormancy has been reported in different varieties (Agrawal, 1981; Siddique *et al.,* 1988; Seshu and Dadlani, 1991; Noredo *et al.,* 1998). Largely rice varieties have seed dormancythat range from 0-11 weeks after harvest, seed dormancy in rice caused some newly harvested rice varieties do not germinate although planted in optimum condition (Ilyas and Diarni, 2007). Sutopo (2002) stated that the dormancy of rice seeds will naturally stop once stored in dry condition for 1-2 months, which is referred to as after-ripening period. But, the after-ripening period differ between species and varieties. Such differences presumably isdue to genetic diversity of dormancy trait among species or varieties of plants.

This can make it difficult for seed analyst to conclude seed germination evaluation when dormancy breaking method used is not effective. In this condition, dormant-seed will be mistakenly classified as dead seed by analyst, thus the test results do not represent the actual condition. In addition, seed analysts sometimes re-testing the sample that suspected in dormant condition more than once to get the result of germination percentage at least 80% (minimum standard of germination for certified seed accepted in Indonesia). This condition can cause delays in distribution of seeds and resulted in limited availability of seeds on the market.

Currently, seed quality is controlled through the certification system or the national standardization system (SSN) (Nugraha, 2007). To get the correct certification decisions, therefore, seed quality testing must be done using methods that have been shown to have an accuracy and precision (reproducibility and repeatability) in accordance with the requirements (International Seed Testing Association, 2010). The low percentage of seeds that passed the laboratory test showed the weakness of the quality assurance system in seed quality testing due to the weakness of the validity of the results of the quality tests in the laboratory with methods that have not been fully accordance with the requirements of the standard method. Standard method for seed testing in laboratory such as ISTA (International Seed Testing Association) recommendation has not been fully implemented, while the substitute method applied have not been through a validation process in accordance with the guidelines ISTA Rules.

The validity of seed testing results through seed germination test will be disrupted by seed dormancy. If at the end of evaluation is still found dormant-seed, then the test should be repeated. According to ISTA Rules (2010) if the results of germination test show the percentage of dormant-seed greater than 5%, then the test should be repeated with seed dormancy breaking applications. Some suggested methods of breaking dormancy by ISTA Rules, among others; drying seeds at temperature of 50^oC, soaking seeds in water or in 1N HNO_3 solution for 24 hours. However, the research results of Soejadi and Nugraha (1992) shows that not all methods of breaking dormancy recommended by ISTA Rules can be applied to rice varieties from Indonesia, for example the application of 1N HNO_3 proved ineffective in breaking dormancy of tested seeds, resulting in all of the seeds even death.

Soejadi and Nugraha (2002) states that the effectiveness of dormancybreaking method is strongly influenced by the dormancybehavior (intensity, persistency, and the mechanism of dormancy).The behavior of seed dormancy varied among genotypes or varieties. Intensity of dormancy is the percentage of dormant seeds at harvest. The higher of the intensity of dormancy indicates that the tested seeds have a low germination rates at harvest. Persistency of dormancy is period of storage (in weeks) needed by seeds from harvest until the moment in which the percentage of dormant-seeds have reached ≤5% stored on ambient storage.

The effectiveness of breaking dormancy method is also influenced by variety (Wahyuni and Nugraha, 2007). Research results ofWahyuni*et al.* (2011) conducted on 20 new-improved varieties of rice newly released, showing all tested varieties responded differently to methods applied. Two methods of breaking dormancy i.e drying seeds in oven at 50^oC for 48 hours continued by soaking seeds in water for 48 hours or in 3% KNO_3 solution for 48 hours were the most effective methods for breaking dormancy. These treatments broke seed dormancy of 19 varieties tested both for fresh and one week after storage. Therefore, to support seed production and quality control, research in rice seed dormancy is required as variety continuously being developed. The availability of effective methods for breaking seed dormancy of rice varieties newly released will help analyst to deliver valid data of germination test.

MATERIALS AND METHODS

Rice Materials

In this experiments, rice seeds were used from 15 varieties planted at Sukamandi Research Station, from November 2011 to March 2012, which have agro-ecological diversity, among others; irrigation rice (Inpari 6, Inpari 6, Inpari 12, Inpari 13, Inpari 14, Inpari 15, Inpari 16, Inpari 18, Inpari 19, Inpari 20, InpariSidenuk), and swampy rice (Inpara 3, Inpara 4, Inpara 6). The seeds were produced from cultivation by applying technique recommended for rice production. Samplings were carried out immediately after harvest for testing the intensity of dormancy and initial vigor in February-March 2012.

Evaluation of Initial Seed Vigor

Initial seed vigor test is to determine the condition of the initial quality of the seeds. Seed vigor revealed by accelerated aging test (AAT) illustrates the percentage of normal seedling after exposed to 45^oC of temperatureand 95% of relative humidity for 120 hours (ISTA, 2010). The experimental design used was completely randomized design (CRD) with three replications. As a comparison, the percentage of normal seedling evaluation at harvest was done without given dormancy breaking

treatment. Analysis of normal seedlings (germination) was conducted using folded-paper method with modification of growing paper (newsprint paper) and incubated in a germinator-room with high temperature and humidity (T=25-30^{o}C, RH=85-90%). Germination was performed at 100 grain of fresh seeds and repeated three times. Observations were made twice, the first observation made on the 5th day count after sowing, and the second observation on the 14th day count.

Evaluation of Seed Dormancy Character

Character dormancy evaluated in this study was the intensity of dormancy and persistency of dormancy. The test was done using CRD with three replications, each of three replicates consist of 100 newly harvested. The analysis of normal seedling was done with a method that has been described as above. The number of dormant-seed was evaluated. The seeds for persistency of dormancy test have been through the process of drying and processing to reach moisture content of ±11%. Then, the seeds were packed in 0.8 mm thick plastic bag, sealed, and stored at ambient storage (T=25-30^{o}C, RH=65-85%). The test was done using CRD with three replications, each of three replicates consist of 100 grain **NEWLY** harvested. The analysis of normal seedling was done with a method that has been described as above. Germination was done from 0 weeks (shortly after the sample was received) until the period of storage when each variety was able to produce normal seedling percentage ≥85%. Interval of germination was done every single week.

Evaluation of Effective Method for Breaking Rice Seed Dormancy

Evaluation method of breaking seed dormancy carried out to obtain one or more effective methods to break seed dormancy of 15 new-improved varieties tested. Samples of seeds already dried to achieve moisture content of ±11%. Prior to germination test, seeds were treated with some treatments for breaking dormancy, among others: (1) soaking seeds in water for 48 hours, (2) soaking seeds in 3% KNO3 for 48 hours, (3) soaking seeds in 10 ppm GA3 for 24 hours, (4) oven-drying seeds at 50oC for 48 hours, (5) oven-drying seeds at 50oC for 48 hours continued soaking seeds in 3% KNO3 for 48 hours, (6) oven-drying seeds at at 50oC for 48 hours continued soaking seeds in water for 48 hours, (7) oven-drying seeds at at 50oC for 48 hours continued soaking seeds in 10 ppm GA3 for 24 hours, and (8) un-treated (control).The treatment wasarranged in a factorial CRD with two factors and repeated three times. The first factor was rice variety with 15 levels, and the second factor was the method of breaking dormancy with 8 levels. The analysis of normal seedling was done with a method that has been described as above.

Statistical Method

Statistical analysis was performed by using SAS Software Release 9.13 for analysis of variance (ANOVA) at 5% level and comparison of means (DMRT) was made at 5% level. Arcsin transformation was conducted before analyzing the data that were not normally distributed (e.g. percentage of germination).

RESULTS AND DISCUSSION

Initial Seed Vigor, Intensity of Dormancy, and Persistency of Dormancy

The intensity of dormancy of 15 new-improved varieties varied between 47-98% (Table 1). Intensity of dormancy illustrates the percentage of dormant-seeds at harvest. In this study, all tested varieties showed the percentage of dormant-seeds >5%. Similarly with germination percentage at harvest showed a value below 85%, which was between 0-39% (Table 1). According to recommendation of ISTA Rules, if the results of germination of dormant-seed greater than 5%, the test should be repeated with dormancy breaking applications. It can be concluded that the tested varieties showed dormancy state at harvest. A total of 13 varieties had the intensity of dormancy varied in a narrow range, which was between 85-96% (Table 1). There were two varieties showed a low intensity of dormancy, Inpari 18 (47%) and InpariSidenuk (60%). The results are consistent with research results of Soejadi and Nugraha (2002) that behavior of seed dormancy, as intensity of dormancy varies between rice genotypes. Takahashi (1995) states that the diversity of intensity of dormancy between varieties is influenced by genetic factor of each variety.

This diversity of intensity of dormancy in 15 new-improved rice varieties suspected due to genetic diversity among varieties.The influence of genetic factor is suspected to be affected by the character of the parent of a new variety. For example, Inpari 14 has high intensity of dormancy (>90%)

and one of the parent of Inpari 14 is Way Apo Buru. Dormancy character shown by Inpari 14 have in common that is owned by its parent. Soejadi *et al.* (2001) reported that Way Apo Buru has intensity of dormancy 96%.Although, seeds were still in dormant condition at harvest, initial quality of fresh seeds used as material research had high quality whereby all varieties had more than 85% of initial vigour, ranged from 87% to 97% (Table 1).

Persistency of dormancy of all tested varieties showed variation between varieties, which was between 2 to 8 weeks (Table 2). Percentage of germination at harvest of each varieties, which was between 0-39% (Table 1) increased at 0 weeks between 1-59% (Table 2), the seeds have undergone the process of drying and processing to reach a safe moisture content for seed storage (±11%). An increase in the percentage of normal seedling (germination) indicating that some seeds broke dormancy during the process of drying and seed processing.

In the second week of storage Inpari Sidenuk (85%) had been able to produce a normal seedling percentage ≥85%. This provide the information that after storage 2 weeks, variety of InpariSidenuknaturally has suffered a broken dormancy. For Inpari 6 (92%) and Inpari 19 (85%), needed 8 weeks of storage time until the two varieties were able to produce a normal seedling percentage ≥85%. At harvest, Inpari Sidenuk(60%) showed the intensity of dormancy lower than 13 other varieties (Table 1).

Table 1. Intensity of dormancy, germination, and initial vigor of 15 new-improved rice varieties at harvest

Variety	*Intensity of Dormancy (%)	Germination (%)	Initial vigor (%)
Inpari 4	98^{a}	0^{c}	94^{a-d}
Inpari 6	98^{a}	1^{bc}	91^{d-f}
Inpari 12	96^{a}	1^{bc}	87^{g}
Inpari 13	93^{a}	3^{bc}	95^{a-c}
Inpari 14	91^{ab}	4^{bc}	93^{b-e}
Inpari 15	92^{ab}	4^{bc}	97^{a}
Inpari 16	89^{ab}	7^{b}	92^{c-e}
Inpari 17	98^{a}	0^{c}	89^{e-g}
Inpari 18	47^{d}	38^{a}	87^{g}
Inpari 19	85^{b}	6^{bc}	88^{fg}
Inpari 20	96^{a}	1^{bc}	96^{ab}
Inpari Sidenuk	60^{c}	39^{a}	95^{a-c}
Inpara 3	92^{ab}	7^{b}	90^{d-g}
Inpara 4	96^{a}	3^{b}	95^{a-c}
Inpara 6	90^{ab}	5^{bc}	93^{b-e}

*) means in each column followed by the same lower case letter are not significantly different (p. 0.05) based on DMRT analysis

Intensity of dormancy illustrates the percentage of dormant-seeds produced until the end of germination. This provide the information that the variety with low intensity of dormancy tends to have a short persistency of dormancy.

Come *et al.* (1988) stated that the differences in persistency of dormancy influenced by several factors, namely species, variety, season, location, and stage of seed development. In this study, diversity of persistency of dormancy among varieties allegedly caused by the genetic diversity among varieties, because the tested seeds were obtained from the same planting location and environment (Sukamandi, Subang) and the same planting time (November 2011 to March 2012).

Soejadi and Nugraha (1991) classified the persistency of dormancy into three groups, ie short persistency (<4 weeeks), medium persistency (4-8 weeks), and long persistency (>8 weeks). Based on research results, it is known that there are two groups of seeds according to the characteristics of persistency of dormancy, varieties with short persistency of dormancy (InpariSidenuk) and varieties

with medium persistency of dormancy (Inpari 4, Inpari 6, Inpari 12, Inpari 13, Inpari 14, Inpari 15, Inpari 16, Inpari 17, Inpari 18, Inpari 19, Inpari 20, Inpara 3, Inpara 4, and Inpara 6).

Table 2. Persistency of dormancy of 15 new-improved rice varieties

Variety	*Normal Seedling (%)									Persistency
	0	1	2	3	4	5	6	7	8	
	---------- week----------									
Inpari 4	6^{f-h}	14^{de}	53^{b}	73^{b}	85^{bc}	95^{a}	92^{c}	100^{a}	100^{a}	4
Inpari 6	1^{h}	2^{g}	3^{h}	6^{i}	12^{h}	48^{f}	71^{h}	76^{d}	92^{b}	8
Inpari 12	90^{e-f}	16^{d}	20^{f}	37^{g}	69^{d}	95^{a}	94^{bc}	100^{a}	100^{a}	5
Inpari 13	7^{fg}	11^{de}	26^{e}	48^{f}	83^{c}	91^{b}	93^{c}	100^{a}	100^{a}	5
Inpari 14	5^{f-h}	9^{d-f}	32^{de}	54^{de}	90^{ab}	93^{ab}	95^{ab}	100^{a}	100^{a}	4
Inpari 15	3^{gh}	5^{fg}	10^{g}	28^{h}	58^{f}	74^{d}	90^{de}	100^{a}	100^{a}	6
Inpari 16	15^{cd}	22^{c}	43^{c}	67^{c}	89^{b}	93^{ab}	93^{c}	100^{a}	100^{a}	4
Inpari 17	2^{gh}	13^{de}	32^{de}	58^{d}	83^{c}	93^{ab}	92^{cd}	100^{a}	100^{a}	5
Inpari 18	44^{b}	44^{b}	49^{b}	57^{d}	64^{e}	75^{d}	87^{c}	100^{a}	100^{a}	6
Inpari 19	19^{c}	25^{c}	33^{de}	46^{f}	62^{e}	74^{d}	83^{g}	83^{c}	85^{b}	8
Inpari 20	9^{ef}	9^{ef}	14^{g}	30^{h}	75^{d}	87^{C}	90^{e}	100^{a}	100^{a}	5
I.Sidenuk	59^{a}	69a	85^{a}	92^{a}	74^{d}	95^{a}	96^{a}	100^{a}	100^{a}	2
Inpara 3	18^{c}	22^{c}	34^{d}	72^{bc}	87^{c}	91^{b}	89^{ef}	100^{a}	100^{a}	4
Inpara 4	12^{de}	12^{de}	26^{e}	49^{ef}	43^{g}	55^{e}	63^{i}	89^{b}	100^{a}	7
Inpara 6	5^{f-h}	10^{d-f}	32^{de}	26^{h}	89^{b}	93^{ab}	94^{bc}	100^{a}	100^{a}	4

*) means in each column followed by the same lower case letter are not significantly different (p. 0.05) based on DMRT analysis

Effective Method for Breaking Rice Seed Dormancy

A method of breaking dormancy is considered effective if after treatment capable of producing percentage of seedling normal ≥85% (Nugraha, 2007). Control seeds of all tested varieties showed percentage of normal seedling were less than 85% (Table 3), so that it can be concluded that all varieties were in dormant state at the beginning of storage. The breaking dormancy treatments significantly giving impact in increasing the percentage of normal seedling of all tested varieties and each variety showed different responses to each treatment were given.

Method 5, 6, and 7 showed the effectiveness of breaking dormancy treatments, because the treatments were able to increase the germination percentage in most number of varieties tested. Those treatments are combination treatments of drying at high temperature and soaking in the solution. Method 7, drying seeds at 50^{o}C of temperature for 48 hours followed by soaking seeds in solution 10 ppm GA_3 for 24 hours showed better response than the other treatments, the dormancy of 14 tested varieties were able to be broken at the beginning of storage. This method can break seed dormancy of Inpari 6 which has high intensity of dormancy (98%) (Table 1) and fairly long persistency of dormancy (8 weeks) (Table 2).

Therefore, the granting of this method can shorten the period of after-ripening for 8 weeks.The results showed that treatments with a combination of drying seeds at high temperature followed by soaking seeds in solution more effective in breaking seed dormancy compared to single treatment. This indicates that the mechanism of seed dormancy occurs in 15 new-improved varieties related to physical properties of seed coat that is impermeable to substances that are needed for germination and the development of the immature embryo.

Bewley and Black (1995) stated that seed dormancy can be caused by the presence of inhibitors in the embryo, or the obstruction of water uptake and oxygen caused the impermeability of seed coat. Causes and mechanisms of seed dormancy is an important thing to be known to determine the way or method to break seed dormancy. The use of high temperature has important role as a stimulator in breaking dormancy that is needed in early stages of germination.

Table 3. The effect of breaking dormancy methods on percentage of normal seedling of 15 new-improved rice varieties

Variety	*Breaking Dormancy Methods							
	1	2	3	4	5	6	7	8
Inpari 4	59	69	70	75	96	95	91	6
Inpari 6	10	24	27	8	80	90	96	1
Inpari 12	15	28	36	46	89	95	93	9
Inpari 13	13	19	33	73	94	96	95	7
Inpari 14	34	33	18	87	97	97	96	5
Inpari 15	17	23	15	47	95	97	86	3
Inpari 16	48	61	34	84	91	98	95	15
Inpari 17	22	52	13	38	96	87	87	2
Inpari 18	57	64	64	74	72	78	88	44
Inpari 19	45	48	46	54	81	79	84	19
Inpari 20	18	36	35	47	95	93	97	9
Inpari Sidenuk	87	89	70	97	93	97	95	59
Inpara 3	22	31	40	33	92	94	89	18
Inpara 4	13	47	35	19	89	77	89	12
Inpara 6	20	75	66	76	92	94	95	5
Effectivity	1	1	-	2	12	12	14	

*)1: soaking seeds in water for 48 hours,2: soaking in 3% $KNO_3$for 48 hours, 3: soakingin 10 ppm GA_3 for 24 hours, 4: drying at 50^oC for 48 hours,5:drying at 50^oC for 48 hours + soaking in3% $KNO_3$for 48 hours, 6: dryingat 50^oC for 48 hours + soaking in waterfor 48 hours, 7: drying at 50^oC for 48 hours + soaking in 10 ppm $GA_3$for 24 hours, and8: control

Martinez-Gomez and Dicenta (2001) reported that in order to improve the effectiveness of the method of breaking seed dormancy by drying at high temperature can be combined with some other treatments such as additional treatment of chemicals solution or growth regulators. In this study, KNO_3solution is applied to the seeds and gave a positive response in increasing the percentage of normal seedling and able to break seed dormancy of some tested varieties.Finkelstein et al. (2008) added that the components of nitrate or nitrite in KNO_3 in addition to stimulate seed germinations,can also stimulate dormancy breaking. The effectiveness of KNO_3 in breaking seed dormancy associated with the increased and availability of O_2 to support the activity of pentose phosphate path, and inhibits oxygen for respiration, inhibits the activity of catalase, thusseed dormancy breaking stimulated and formed normal seedling.

Hartman et al. (2011) stated that seed dormancy can be caused by the presence of germination inhibitors (abscisic acid) that the proportion is not balanced with growth regulators (gibberellin). On dormant-seed, the level of gibberellin acid is still low. In this study, the addition of exogenous gibberellin acid was done to raise the level of GA_3 in order to stimulategermination,it is suspected that exogenous gibberellin can affect the physiological changes in the seed, such as maturation of the embryo which is as a response to plant growth regulators.

CONCLUSIONS

In this study obtained three effective methods of breaking seed dormancy, (1) drying seeds at 50^oC for 48 hours continued by soaking seeds in 10 ppm GA_3 for 24 hours, (2) drying seeds at 50^oC for 48 hours continued by soaking seeds in water for 48 hours, and (3) drying seeds at 50^oC for 48 hours continued by soaking seeds in 3% KNO_3 for 48 hours. It is hoped the methods can be used as substitute and standardized methods that can be applied in seed quality testing.

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