Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 5(8) pp. 325-331, August, 2016 Issue.
Available online http://garj.org/garjas/home

Full Length Research Paper

The use biological agent *Pseudomonas fluorescens* P60 of seed health, physiological quality, and growth of Seedling IR 64

Latarus Fangohoi[1], Lisa Navitasari[1] and Achmad Nizar[1]

[1]Colleges of Agricultural Extension, Malang, Indonesia.

Accepted 1 August, 2016

The purpose of the research were: (1) test the IR 64 seed health, (2) test *P. flourescens* P60 in improving seed health, (3) test *P. Fluorescents* P60 on physiological seed quality, (4) test *P. flourescens* P60 on the growth of seedlings in a greenhouse. The results showed IR-64 infected seed borne pathogens of *Aspergillus flavus*, *Alternaria padwickii*, *Pseudomonas glumae* and *Pseudomonas syringae*. Testing of *P. flourescens* P60 in improving seed health, indicating *P. flourescens* P60 could improve seed health by inhibiting the growth of the colony *A. padwickii*, *A. flavus*, and *P. glumae*, but it did not inhibit the growth of colonies of P. Syringae. While testing *P. flourescens* P60 to physiological quality of seeds, *P. flourescens* P60 was able to maintain the physiological quality with germination above 80%. The results in the greenhouse showed that *P. flourescens* P60 with soaking time 15 minutes and 25 minutes had the same effect on seedling height, root length and seedling dry weight. However, 25 minutes soaking time resulted in heavy wet seed and root dry weight were higher than soaking time of 15 minutes.

Keyword: *Pseudomonas fluorescent* P60, seed health, physiological quality, growth of seedling of IR 64.

INTRODUCTION

IR 64 has been released by IRRI in 1984 and it is still widely used today. However, the availability of improved seed is becoming an obstacle due to seed quality continues to decline, either seed health and physiological quality of the seed. The seed borne pathogen is one of the causes of low seed health, which result in low physiological quality of seeds, including seeds failed to germinate and sprout growth is not normal (Agarwal and James, 2000; Sutopo, 1993), thus resulting in the need for seed treatment.

Seed treatment is generally carried out physically and chemically. Physical seed treatment such as soaking seeds before planting, whereas chemical seed treatment using a gas such as methyl bromide, phosphine, Florida

*Corresponding Author's Email: lissa_nav@yahoo.com

mercury compound, liquid organic mercury, and other fungicides (Agarwal and James, 2000). The use of synthetic chemicals in seed treatment resulted in a decrease in seed viability and shortens the life span of the seed, so it needs another alternative in the treatment of seeds, one of them using a biological agent *Pseudomonas flourescens* P60 that is antifungal and antibacterial. *Pseudomonas flourescens* P60 is a biological agent that is isolated from the roots of wheat plants (Soesanto *et al.,* 2011) and it is widely used as a biological control of soil-borne pathogens (Santoso *et al.,* 2007). However, the potential of *P. flourescens* P60 has never been used in seed treatment to suppress the presence of seed-borne pathogens. The research aims to (1) test the IR 64 seed health, (2) test *P. flourescens* P60 in improving seed health, (3) test *P. Fluorescents* P60 to physiological seed quality, (4) test *P. flourescens* P60 on the growth of seedlings in a greenhouse.

MATERIALS AND METHODS

Time and Place Research

The study was conducted in September 2012-March 2013 at the Centre for Development and Empowerment of Teachers and Education Personnel of Agriculture (PPPPTK) Cianjur, General Soedirman University (UNSOED) Purwokerto, and Research Center of Biology-LIPI, Cibinong, Indonesia.

Seed health

Seed health tested by blotter test method. Seeds were grown in Petri dishes lined with wet paper stencil. Seeds were incubated at room temperature with NUV(Near Ultra Violet) irradiation for 12 hours of light and 12 hours dark by turns, then for 24 hours at -20 ^{0}C. On the third day, the seed re-incubated at room temperature under irradiation NUV 12 hours of light and 12 hours dark alternately until the seventh day (ISTA, 2008). On the eighth day,the seeds were observed and identified pathogens with identification keys Pictorial atlas (Watanabe, 1994),and they were calculated the percentage of pathogen attack. The formula was $\frac{\sum \text{Infected seeds}}{\sum \text{total seed}}$ X 100%, and then the seeds were isolate of the pathogen with single spore isolation technique (Turechek and Stevenson, 1998).

Testing of *Pseudomonas flourescens* P60 to seed health.

This test used blotter test method. Detection of pathogens carried by disinfected with NaOCl1% and without NaOCl 1%. Seeds were incubated at room temperature with NUV irradiation light for 12 hours, then for 24 hours at -20^{0}C. The third day, the seed re-incubated under NUV irradiation 12 hours of light and 12 hours dark alternately until the seventh day (ISTA, 2008). Eighth day, the seeds were observed, and identificate of seed-borne pathogens with identification keys Pictorial atlas (Watanabe, 1994), and they were calculated the percentage of pathogen attack by the formula $\frac{\sum \text{Infected seeds}}{\sum \text{total seed}}$ X 100%,

Testing of *Pseudomonas flourescens* P60 against physiological seed quality.

Seed germination

Seed germination test use rolled paper on top of the plastic. Germination was calculated on the 7th day as count I (KN I) and 14th day as count II (KN II) (ISTA, 2004; Wongvarodom and Naulkong, 2006), and they were calculated the percentage of germination = $\frac{\sum KN\ I + \sum KN\ II}{\sum \text{total seeds}}\ X\ 100\ \%$

Seedling dry weight

Normal seedling on 14th day after planted was removed from the seeds / cotyledons were still attached and then dried in an oven at a temperature of 80^{0}C for 24 hours, then the seedling put in a desicator ± 30 minutes and weighed (ISTA, 2004), seedling dry weight was calculated K1 - K0. K1 was the initial weight of the germination before in the oven and K0 was the final weight after germination in the oven.

The growth rate of germination

The growth rate of germination was a method developed by Burris (1976 in Copeland and McDonald, 2001). Benchmark was calculated by dividing the normal seedling dry weight by the number of abnormal germination dried in an oven at 60^{0}C for 3x24.

The rate of growth of the sprouts was $\frac{BKKN}{\sum KN}$ X 100 %.

Maximum growth potential

Maximum growth potential was calculated based on the percentage of seeds capable of germination of normal or abnormal on the last day of observation (14th day) per number of seeds planted (ISTA, 2004), and it was calculated the percentage : $\frac{\sum \text{growing seed}}{\sum \text{total seeds}}$ X 100 %.

Speed of growth

Speed of growth was measured by counting the germination grow normally. Each observation of normal seedling number divided by 24 hours (t), which was calculated from the time, when the seed was planted until the last observation (N) (Sadjad, 1993), and then the seed was calculated the percentage growth by using the formula :$\sum_0^{tn} \frac{N}{t}$.

Vigor Index (VI)

Vigor index was calculated based on the percentage of normal seedling on the first count (7th day) (ISTA, 2004), it was calculated the percentage of vigor index (IV) : $\frac{\sum KN\ I}{\sum Total\ seed}$ x 100 %.

Testing *Pseudomonas flourescens* P60 on the growth of IR 64 seedlings in the greenhouse.

Seeds treated with *P. flourescens* P60 planted in polythene bags containing 300 g of soil that had been pasteurization, fertilized with specific suggestions Cianjur. Each polybag planted seeds of five grains of IR 64 and 3 repeated, observed seedling height, root length, wet weight of seed, seedling dry weight, and root dry weight (Agustiansyah *et al*., 2010).

Analysis of IAA in *Psudomonas flourescens* P60.

IAA qualitative analysis. Bacteria that produce IAA tested qualitatively by colorimetric method using Salkowski reagent (Gordon and Weber, 1951).

IAA analysis quantitatively. IAA concentration was counted by HPLC method. The first step was by taking 2 mL liquid culture of bacteria in centrifuged at 12,000 rpm for 10 minutes, and then the supernatant was taken injected on HPLC. In addition mobile phase was water. It used concentration water and acetonitrile (76:24 v/v). Finally it filed in columns18. Reverse phase calculated by the UV detector at a wavelength of 280 nm, meanwhile the maximum temperature column was kept on 32.6 ºC with a pressure of 10, 9 MPa. The result of AA concentration of the sample was calculated based on a standard curve with pure IAA standard (Tien *et al.,* 1979; Lee, *et al.,* 2004; Patil, *et al.,* 2011).

Data analysis

The data were analyzed for analysis of variance (ANOVA) using the Statistical Analisys System (SAS 2003). Each treatment significantly was tested by Duncan's multiple range test at α = 0.05 (Mattjik and Sumertajaya, 2006).

RESULTS AND DISCUSSION

IR 64 Seed Health

IR 64 seed health testing showed the presence of infection-seed-borne pathogens in IR 64 were *Aspergillus flavus*, *Alternaria padwickii*, *Pseudomonas glumae*, and *Pseudomonas syringae* (Figure 1).

Testing *Pseudomonas flourescens* P60 on seed health.

Testing of *P. flourescens* P60 on seed health not only showed significant effect of pathogen *A. flavus, A. padwickii*, but also *P. syringae* significantly effected on benomil treatment, seed sources and disinfectant. In contras disinfectan treatment of *P.glumae* showed no effect of real. It is assumed the *P.glumae* is in seed tissue. Cottyn (2002) reported *P.glumae* is generally located in the seed tissues (grain), so seed surface of disinfectant does not reduce the level of pathogen attack.

Disinfestation kill fungi, bacteria, or insects that are in the seed surface (surface organism) (Desai *et. al*., 1997). The real effect between *P. flourescens* P60 and benomil treatment attack pathogen, because P60 has antibiotics and siderophores. It supported by Susanto *et. al.* (2011). They have anti-fungal and anti-bacterial. While benomil have karbendazim active compounds that toxic and broad-spectrum fungicide (Syahbirin *et al.,* 2001). Table 1 showed the treatment of *P. flourescens* P60 attack pathogens significantly different from aquadest, and *P. flourescens* P60 able to suppress seed-borne pathogens. In other words the treatment of *P. flourescens* P60 able to suppress levels of pathogens *A.flavus, A.padwickii, P. syiringae*, and *P.glumae,* because of biochemical characteristics possessed *P. flourescens* P60 as antibiotics and siderophores (Susanto *et al*., 2011). Dwivedi and Johri

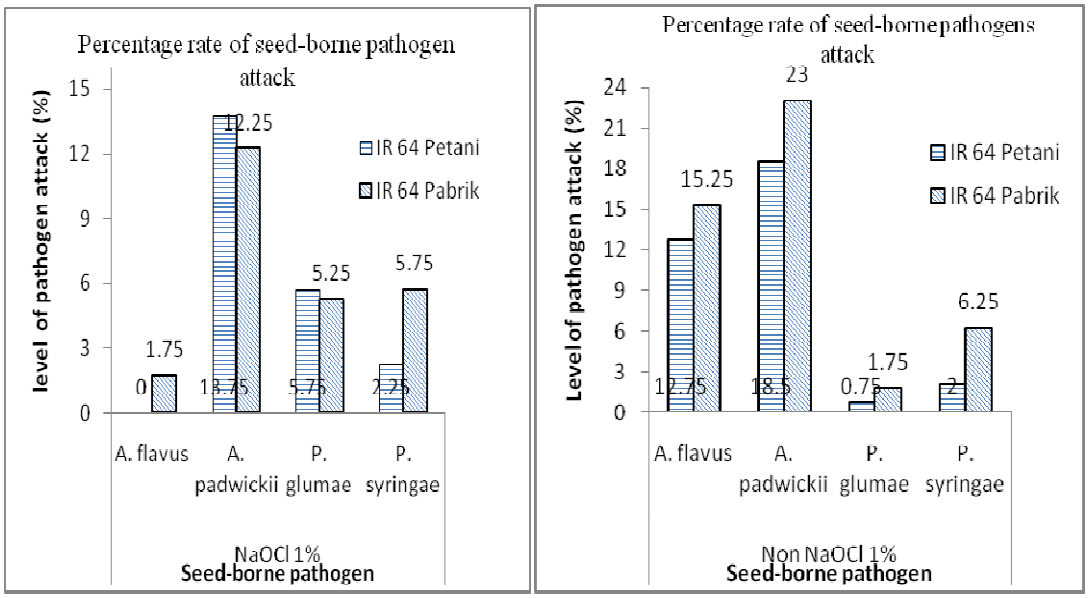

Figure 1: The percentage of seed-borne pathogens attack IR 64

Table 1: Level seed-borne pathogens attack on a variety of treatments

Treatment	Level seed-borne pathogens (%)			
	A. flavus	***A. padwickii***	***P. syringae***	***P. glumae***
P605	0,3 b	8,7 b	0,7 bc	0,9 bc
P6015	0,3 b	6,8 b	1,3 b	0,2 c
P6025	0,2 b	9,1 b	1,4 bc	0,6 bc
P6040	0,4 b	8,7 b	0,8 bc	1,2 ab
Aqua15	7,3 a	16,4a	4,1 a	2,4 a
Beno5	0,2 b	4,6 c	0,4 c	2,4 a
NaOCl	0,4 b	6,4 b	1,1 b	1,2 a
NotNaOCl	2,5 a	11,7 a	1,8 a	1,3 a

Note: Numbers followed the same letter in the same column indicates not significantly different according to Duncan test at α = 0.05. P605 = *P. fluorescents* P60 soaking 5 minutes; P6015 = *P. fluorescents* P60 soaking for 15 minutes; P6025 = *P. fluorescents* P60 soaking 25 minutes; P6040 = *P. fluorescents* P60 soaking 40 minutes; Aqua15 = aquadest soaking 15 minutes; Beno5 = benomil soaking 5 minutes.

(2003) reported, antibiotics pyoluteorin (PLT), pyrrolnitrin (Prn), phenazine-1-carboxylic acid (PCA) and 2,4-di-acetylphloroglucinol (PHL) is an antifungal and antibacterial.

Effect of *Pseudomonas flourescens* P60 to physiological seed quality

The treatment of *P. flourescens* P60 and benomil treatment did not significantly effect germination, growth potential maximum, speed of germination grow, and vigor index, but

Table 2: Results of physiological quality testing on a variety of treatments

Treatment	Physiological quality					
	Seed Viability			Seed Vigor		
	DB (%)	BKC (g)	LPK (%)	PTM (%)	KCT (%/24 hours)	IV (%)
P605	82,3 a	0,3 bc	82,2 abc	82,3 a	1,7 a	75,0 a
P6015	85,3 a	0,4 bc	81,0 bc	85,3 a	1,8 a	77,0 a
P6025	81,7 a	0,3 bc	84,0 abc	81,7 a	1,7 a	77,7 a
P6040	82,0 a	0,3 c	78,0 c	82,0 a	1,7 a	75,3 a
Aqua15	82,0 a	0,4 ab	87,8 ab	82,0 a	1,7 a	78,0 a
Beno5	85,7 a	0,4 a	88,2 a	85,7 a	1,8 a	80,0 a

Note: Numbers followed the same letter in the same column indicates not significantly different according to Duncan test at α = 0.05. P605 = *P. flourescens* P60 soaking 5 minutes; P6015 = P60 soaking for 15 minutes; P6025 = P60 soaking 25 minutes; P6040 = P60 soaking 40 minutes; Aqua15 = aquadest soaking 15 minutes; Beno5 = benomil soaking 5 minutes. DB = germination; BKC = seedling dry weight; LPK = Growth rate of germination; PTM = Potential maximum growth; KCT = Speed germination grow; IV = vigor index.

Table 3: The results testing of seedling growth in a variety of treatment for 21 days after planting in the greenhouse

Treatment	Components of Seed Growth				
	TB (cm)	PA (cm)	BBB (g)	BBK (g)	BAK (g)
P6015[2)]	32,8 ab	12,4 a	3,9 a	0,7 ab	0,2 a
P6025	33,9 a	12,8 a	4,2 a	0,8 a	0,2 a
Aqua15	32,1 b	11,9 a	3,1 b	0,7 b	0,2 a
Beno5	32,5 ab	12,3 a	3,2 b	0,7 ab	0,1 b

Note : [1)] Numbers followed the same letter in the same column indicates not significantly different according to Duncan test at α = 0,05.[2)] P6015 = P60 soaking for 15 minutes; P6025 = P60 soaking 25 minutes; Aqua15 = aquadest soaking 15 minutes; Beno5 = benomil soaking 5 minutes. 3) TB = high seed; PA = root length; BBB = weight of wet seeds; BAK = weight of dry seeds; BAK = weight dry root.

Table 4. Results of analysis IAA *Pseudomonas fluorescents* P60 using high performance liquid chromatography (HPLC).

Sampel *Pseudomonas flourescens* P60	Contens of IAA (ppm)
Original	2,97
Dilution to-8 (Formula application)	2,48

Note: Diluent used is 0.85% NaCl saline

They significantly differ to the dry weight germination and germination growth rate. This indicates that the treatment of *P. flourescens* P60 has the same effect with benomil on viability and vigor, germination rate above 80%. Raka *et al*., (2012) reported that the total value of the variable viability and potential viability above 80%. This is a very informative value for seed producers. According to ISTA (2004), the seeds has a good physiological quality if germination above 80%.

The treatment of *Pseudomonas fluorescents* P60, and benomil showed a significantly effect on root dry weight and seed weight wet (Table 3), but both treatment not significantly influence on seedling height, root length, and seedling dry weight.This is caused by *P. flourescens* that produce *Indole-3-acetid acid* /IAA (Table 4) and dissolving phosphate (Figure 2).

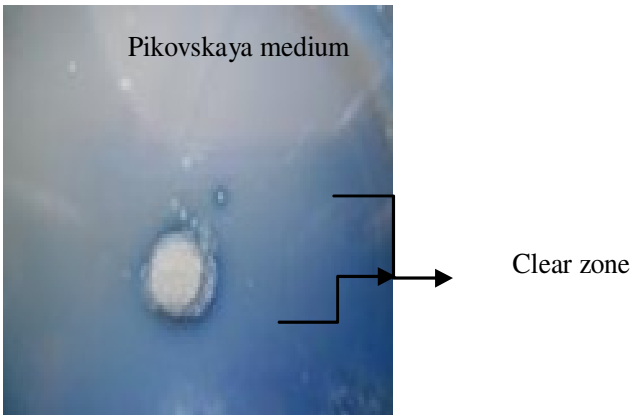

Figure 2: *Pseudomonas fluorescents* P60 as solvent microbial phosphate (MPF)

CONCLUSION

1. IR 64 has been infected seed-borne pathogens *Aspergillus flavus*, *Alternaria padwickii*, *Pseudomonas glumae*, and *Pseudomonas syringae*.
2. Testing of *Pseudomonas fluorescents* P60 on seed health showed decrease the percentage level of seed-borne pathogens attack.
3. Testing of *Pseudomonas flourescens* P60 on physiological quality showed *P. flourescens* P60 able to maintain the physiological quality of the germination rate above 80%.
4. The treatment of *P. flourescens* P60 with soaking time 15 minutes and 25 minutes in green house have the same effect on seedling height, root length, and seedling dry weight. However, the treatment of 25 minutes soaking of seeds produce wet root weight and dry root weight higher than 15 minutes soaking.

ACKNOWLEDGEMENT

The authors acknowledge the support provided by the laboratory of seed technology and tissue culture, Department of Agronomy, University General Soedirman (UNSOED) Purwokerto, and Research Center of Biology-LIPI, Cibinong, Indonesia

REFERENCES

Agarwal VK, dan James BS (2000). *Principles of Seed Pathology*. Vol. II. CRC Press. Boca Raton, Florida.

Agustiansyah S, Ilyas, Sudarsono, dan Machmud M (2010). Pengaruh Perlakuan Benih secara Hayati pada Benih Padi Terinfeksi *Xanthomonas oryzae* pv. *oryzae* terhadap Mutu Benih dan Pertumbuhan Bibit. *Jurnal Agronomi Indonesia*. 38 (3):185–191.

Copeland LO, McDonald MB (2001). *Principle of Seed Science and Technology*. 4th ed. Kluwer Academic Publisher. Massachusetts. 467p.

Cottyn B (2002). Bacteria associated with rice seed from Philippine farmers fields. *Tesis*. University of Ghent. Los Banos, Laguna. http://dspace.irri.org:8080/dspace/bitstream/123456789/446/1/CDPDF2002.Cottyn,B.pdf. Diakses tgl 5 Maret 2013.

Desai BB, Kotecha PM, Salunkhe DK (1997). *Seeds Handbook: Biology, Production, Processing, and Storage*. Marcel Dekkerm, New York.

Dwivedi D, Johri BN (2003). Antifungals from Flourescent Pseudomonads : Biosynthesis and Regulation. *Current Science*. 85(12):1693-1703.

Gordon SA, Weber RP (1951). Colorimetric estimation of indoleacetic acid. *Plant Physiology*. 26:192-195.

International Seed Testing Association (ISTA). (2004). *International Rules for Seed Testing*. Zurich. International Seed testing Association.

International Seed Testing Association (ISTA). (2008). International Rules for Seed Testing, Annexe to Chapter 7: *Seed Health Testing Methods*. Bassersdorf, Switzerland. Hal 7-11.

Lee S, Flores-Encarnaclon M, Contreras-Zentalla M, Garcia-Flores L, Escamilla JE, Kennedy C (2004). Indole-3-acetic acid biosynthesis is deficient in *Gluconacetobacter diazotrophicus* strains with mutations in cytochrom biogenesis genes. *J. Bacteriol.* 186 (16):5384-5391.

Mattjik AA, dan Sumertajaya IM (2006). *Perancangan Percoban, dengan Aplikasi SAS dan Minitab*. IPB Press. Bogor.

Maude RB (1996). *Seedborne Diseases and Their Control, Principles and Practice. Horticulture Research International*. Cambrige. CAB International. Hal. 1-26.

Patil NB, Gajbhiye M, Ahiwale SS, Gunjal AB, Kapadnis BP (2011). Optimization of Indole 3-acetic acid (IAA) production by *Acetobacter diazotrophicus* L1 isolated from Sugarcane. *Journal of Environmental Science*. 2(1):295-302.

Raka IGN, Astiningsih AAM, Nyana IDN, dan Siadi IK (2012). Pengaruh *dry heat treatment* terhadap daya simpan benih cabai rawit (C*apsicum frutescens* L.).*Journal Agric. Sci. and Biotechnol.* 1(1):1-11.

Sadjad (1993). *Dari Benih kepada Benih*. Gasindo. Jakarta. Hal 144.

Santoso SE, Soesanto L, dan HaryantoTAD (2007). Penekanan hayati penyakit moler pada Bawang Merah dengan *Trichoderma harzianum*, *Trichoderma koningii* dan *Pseudomonas flourescens* P60. *Jurnal Hama Penyakit Tumbuhan Tropika*. 7(1):53-61.

Soesanto L, Mugiastuti E, dan Rahayuniati RF (2011). Biochemichal Characteristic of *Pseudomonas flourescens* P60. *Journal of Biotechnology and Biodiversity*. 2:19-26.

Sutopo L (1993). Teknologi Benih. PT Raja Gafindo Persada. Jakarta.

Syahbirin G, Purnama H, dan Prijono D (2001). Residu pestisida pada tiga jenis buah impor. *Buletin kimia*. 1:113-118.

Tien TM, Gaskins MH, Hubbel DH (1979). Plant growth subtances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied Environmental Microbiology*. 37(5):1016-1024.

Turechek WW, Stevenso KL (1998). Effects of host resistance, temperature, leaf wetness, and leaf age on infection and lesion development of pecan scab. *Phytopathology*. 88:1294-1301.

Watanabe T (1994). *Pictorial Atlas of Soil And Seed Fungi, Morphologies of Cultured Fungi and Key to Spesies*. Boca Raton, Florida. CRC Press, Inc. Hal. 21-34.

Wongvarodom V, Naulkong S (2006). Responses of bambara goundnut seed to accelerated aging. *Nature Science*. 40:848-853.