IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 10, Issue 10 Ver. IV (October 2017), PP 43-51
www.iosrjournals.org

In Vitro Selection Of Embryogenic Callus To Produce Resitant Peanut Plant Against On Various Races Of Sclerotium Rolfsii In Lombok Island

A. Farid Hemon[1)], Sumarjan[1)], Hanafi AR[1)]
[1)] *Study Program Agroecotechnology Faculty of Agriculture Mataram University Indonesia*
e-mail: faridhemon_1963@yahoo.com

Abstract: *The objective of this study was to produce the variant of embryogenic callus (somatic embryo=SE) which was resistant to various culture filtrate (CF) media of S. rolfsii races and produced resistant peanut lines to S. rolfsii races infection. This research was inisiated of characterized and anastomosis group testing of S. rolfsii from the field collection with mycelial compatibility groups. The next step were development of embryogenic calluses population, making of CF media of S. rolfsii and in vitro selection, regeneration of resistant embryogenic callus, seed production of R1 generation, and evaluation of somaclone peanut against infection of various S. rolfsii races. Result of study showed that 1) embryogenic callus selected in a selection medium containing CF more than one S. rolfsii race generated a percentage of the number of live ESs and the number of ES per explant fewer than embryogenic callus that was selected in CF media containing one race; 2) peanut somaclones produced from embryogenic callus selection on media containing CF various races of S. rolfsii had different responses to several races of S. rolfsii infection. The somaclones population derived from embryogenic callus selected in the selection medium containing more than one race CF resulted in plant damage due to S. rolfsii infection greater than the peanut somaclones derived from embryogenic callus which were selected only on one race CF medium. Overall, somaclones derived from embryogenic calli were selected on selective media containing CF more than one race the S. rolfsii were more resistant from infection of various S. rolfsii races compared with somaclones derived from embryogenic callus that were selected only on selective medium containing CF of one race S. rolfsii.*
Keywords: *somaclonal variation, in vitro selection, S. rolfsii.*

Date of Submission: 02-11-2017 Date of acceptance: 28-10-2017

I. Introduction

Peanut cultivation is more directed at rained irrigated land and dry land. Peanut planting in upland often faced with water defisit constraint mainly in dry season. Global warming in recent years have added this problem especially in peanut farming in dry land. Global warming will be a direct impact on metabolism plants, temperatures and humidity of growing environment and also influenced the development pathogens (Cazares *et al*. 2011).

Another problem faced in the peanut cultivation in dry land is the existence of attacks of various pathogens and the common is stem rot disease caused by fungal infection of *Sclerotium rolfsi*. Stem rot disease caused by *S. rolfsii* infection is the limiting factor of peanut farming production in dry land. This pathogen infection can decrease the quantity and quality of peanut yields. According to Backman and Brenneman (1997) that the decline in peanut crop yield due to *S. rolfsii* attacks can reach 25-80%. *S. rolfsii* is a soil borne fungus and this fungus is capable to forming sclerotia which caused the fungus to long survive and colonize in the soil. Dry land conditions that are difficult to apply irrigation system, causing inoculum fungus difficult to remove on dry land farming, so that inoculum always located throughout in the peanut growing season. In addition, this pathogen is able to form new genetic variability (physiology races) and each race is different pathogenicity to peanut crop (Punja 1985; Benhamou & Chert 1996). The large number of physiological races, causing the difficulty of controlling this pathogen if only relying solely on single-resistant genes. This pathogen attacks can lead to reduced production and crop failure.

The use of a broad spectrum resistant cultivar is a practical and economical alternative to improving the yield of peanuts. In Indonesia, peanut germplasm that are resistant to stem rot disease does not yet exist (Yusnita & Sudarsono, 2004), so that conventional crosses with other high yielding cultivars can not be performed. Therefore, provision of peanut germplasm that has resistance to stem rot diseases is very important.

Efforts to obtain peanut germplasm can be done by increasing the genetic variability of plants through somaclonal variation and followed by in vitro selection of embryogenic callus (Karp 1995; Matsumoto *et al.* 1995).

In vitro cultures and selection of embryogenic callus at the cell and tissue level with using selecting agents are expected to obtain the desired character (Jain 2001). Selection of embryogenic callus may be performed by using a culture filtrate issued by *S. rolfsii* as a screening agent. Media containing culture filtrate of *S. rolfsii* can be used to identify cells or tissues of peanut crops that not killed by culture filtrate (Yusnita *et al*. 2005; Matsumoto et al., 1995). Fungi of *S. rolfsii* secreted large amount of oxalic acid, a phytotoxin responsible for killing cells/tissues, prior to mycelial growth on the infected plant (Porter *et al*., 1982; Backman 1984; Punja 1985). This phytotoxin could be used to develop *S. rolfsii* tolerant peanut lines through *in vitro* selection. Cells or tissues that do not die because the culture filtrate is expected to develop into plants that are resistant to *S. rolfsii* infection.

The result of previous research that in vitro selection of embryogenic callus (somatic embryo=SE) for disease resistance was common in one pathogen and does not distinguish its physiological races, so that the resistant peanut lines that was generated from this research was easier to breaking resistance and become vulnerable to new races (Hemon, 2006). Field observations also that these peanut lines were particularly more susceptible to stem rot disease (Hemon, 2009). Therefore, this research had been done to produce the variant of embryogenic callus (somatic embryo) which was resistant to various culture filtrate media of *S. rolfsii* races and produced resistant peanut lines to *S. rolfsii* races infection.

II. Materials and Methods

Preparation of *S. rolfsii* Races

The *S. rolfsii* fungus races have been isolated from various regions and plant hosts. The various isolates that have been collected then characterized for colony growth on PDA media. After characterization of colony growth, mycelial compatibility groups (MCG) was tested based on modification method developed by Carling et al. (1994) and Bains & Bisht (1995). In steril petridish containing PDA media were placed three colonies of different isolates. The colony diameter used was 5 mm and 3 days old. Distance between colonies was 3 cm. Various isolates were then observed in MCG. Mycelium growth among isolates that incompatibility was considered as a race that was different among tested races (a individual physiological race group).

The results of characterization of colony growth and MCG testing were obtained by four races of *S. rolfsii*, namely race 1, race 6, race 8, and race 9. After obtaining 4 races *S. rolfsii*, then the next testing was pathogenicity test. The result of pathogenic test showed that the four races had different pathogenic to peanut cultivars (data not showed). Four races of *S. rolfsii* were then cultured on PDA media as material for the purposes of making culture filtrate media from various races. The cultural filtrate (metabolite toxin of *S. rolfsii*) was prepared by growing four races of pathogens in MS0 media. At 14 days after planting, the fungus was sterilized to obtaining the culture filtrate (FK).

Development of embryogenic calluses population, making of culture filtrate media of *S. rolfsii* and in vitro selection

Embiogenic callus (KE) was initiated by culturing embryonic leaflets isolated from mature seeds of M4 generation of peanut mutan line. Induction of KE was done in MS medium (Murashige & Skoog 1962) with added 16 µM picloram (MS-P16 medium), mixture vitamins and amino acids (glycine, thiamine, pyridoxine and niacin) 0.1 mg /L, sucrose 2%, and agar 8 g /L.

Selection media of culture filtrate *S. rolfsii* was prepared with mixed basic media MS (Murashige and Skoog, 1962), vitamin B5 (Gamborg et al. 1968), sucrose sugar (30 g / L), agar (8 g/L), and culture filtrate of *S. rolfsii* with a concentration of 35% (Hemon et al., 2017). The culture media was set to a pH of 5.6 before sterilization. After the agar was dissolved by heating, the medium was poured in a 150 mL culture bottle each of 25 mL and covered with plastic. The prepared media was sterilized by autoclave heating at 121° C and 15 psi pressure for 20 minute. After cooling, the culture filtrate media was readily used for in vitro selection testing of embryogenic calluses.

The embryogenic calluses include somatic embryos (SE) were then grown on a selective medium of culture filtrate *S. rolfsii*. The five explants of embryogenic callus, with 10-12 SEs per bottle grown in selective medium containing *S. rolfsii* race 1 (MS-P16-FK-1). Total explants evaluated per selective medium were 500 embryogenic callus or 4000 ESs. Resistant embryogenic calli on MS-P16-FK-1 media were proliferated in MS-P16 medium without the culture filtrate *S. rolfsii* and these resistant embryogenic calluses were called as **KE-Race-1.** The KE-Race-1 embryogenic calluses were subsequently replanted on selective medium containing culture filtrate *S. rolfsii* of race 6 (MS-P16-FK-6). Embryogenic calluses that were resistant on culture media MS-P16-FK-6 proliferated in MS-P16 medium without a culture filtrate *S. rolfsii* and these resistant embryogenic calluses were called as **KE-Race 1-6**. Other embryogenic calluses were grown in MS-P16-FK-6

selective medium and subcultured. The embryogenic callus and SE that were resistant to the MS-P16-FK-6 medium were proliferated in MS-P16 medium without the *S. rolfsii* culture filtrate and these resistant embryogenic calluses were called as **KE-Race-6**.

The KE-Race-1-6 embryogenic calluses were subsequently replanted on the MS-P16-FK-8 selective medium. The resistant embryogenic calluses and ES on MS-P16-FK-8 were proliferated in MS-P16 medium without *S. rolfsii* culture filtrate and these resistant embryogenic calluses were called as **KE-Race 1- 6-8**. Other embryogenic calluses were grown in MS-P16-FK-8 selective medium. The embryogenic calluses that were resistant to MS-P16-FK-8 were proliferated in MS-P16 medium without the culture filtrate *S. rolfsii* and these resistant embryogenic calluses were called as **KE-Race-8**.

The KE-Race-1-6-8 embryogenic calluses were subsequently replanted on the MS-P16-FK-9 selective medium. The resistant embryogenic calluses on MS-P16-FK-9 were proliferated in MS-P16 medium without the culture filtrate S. rolfsii and these resistant embryogenic calluses were termed **KE- 1-6-8-9**. Other embryogenic calluses were grown in MS-P16-FK-9 selective medium. The embryogenic calluses that were resistant SE to MS-P16-FK-9 were proliferated in MS-P16 medium without culture filtrate *S. rolfsii* and these resistant embryogenic calluses were termed as **KE-Race-9** (Table 1).

Table 1. Code name of embryogenic callus population that were resistant to selective media containing culture filtrate of various *S. rolfsii* races

Selective media containing culture filtrate	Population of embryogenic callus that was used	Population of embryogenic callus that was generated
Culture Filtrate Race 1 (MS-P16-FK-1)	Embryogenic callus	Resistant to race 1 (KE-Race-1)
Culture Filtrate Race 6 (MS-P16-FK-6)	Resistant embryogenic callus race 1 (KE-Race-1)	Resistant to race 1 & 6 (KE-Race-1-6)
	Embryogenic callus	Resistant to race 6 (KE-Race-6)
Culture Filtrate Race 8 (MS-P16-FK-8)	Resistant embryogenic callus race 1 & 6 (KE-Race-1-6)	Resistant to race 1, 6, & 8 (KE-Race-1-6-8)
	Embryogenic callus	Resistant to race 8 (KE-Race-8)
Culture Filtrate Race 9 (MS-P16-FK-9)	Resistant embryogenic callus race 1, 6, & 8 (KE-Race-1-6-8)	Resistant to race 1, 6, 8 & 9 (KE-Race-1-6-8-9)
	Embryogenic callus	Resistant to race 9 (KE-Ras-9)

Regeneration of resistant embryogenic callus to culture filtrate and seed production of R1 generation

The resistant embryogenic callus to culture filtrate race 1 (KE-Race-1), resistant to races 1 and 6 (KE-Race-1-6), resistant to race 6 (KE-Race-6), resistant to race 1, 6, and 8 (KE-Race-1-6-8), resistant to race 8 (KE-Race-8), resistant to race 1, 6, 8 and 9 (KE-Race-1-6-8-9), and resistant to race 9 (KE-Race-9) were further germinated to form the planlet KE Race -1, KE-Race-1-6, KE-Race-6, KE-Race 1-6-8, KE-Race-8, KE -Race-1-6-8-9, and the planlet KE-Race-9. Germination process was done in MS medium with active charcoal addition (1 g/L).

These planlets were then regenerated became R0 plant and planted in plastic pots containing 9 kg of sand-soil mixtures, and maintained in a greenhouse until harvest. Seeds R0: 1 harvested from each plant R0 was replanted to produced the R1 generation peanut somaclone plant. Plant somaclones of R1 generation were grown with the same as procedure plant R0.

Evaluation of somaclone plants against infection of various *S. rolfsii* races

The plants material used in this experiment were peanut somaclones population of generation R2 derived from R1 somaclone generation. Seeds of peanut plant that were not exposed under in vitro selection were also used as control plant. The peanut seeds of R2 generation were grown 2 seeds per polybag containing soil medium. At 14 days old, it was left a plant per polybag.

Evaluation of peanut somaclones to *S. rolfsii* infection was inisiated with subcultured pure isolate of four races (1, 6, 8, & 9) *S. rolfsii* in PDA medium. Pure isolate of *S. rolfsii* 14 days old was used as inoculum. Pathogen inoculation to plant was done at 30 days old plant. Agar block (0.5 x 0.5 cm^2) containing pure isolate of *S. rolfsii* was pasted on stem basal of plant and pile up of soil to stem basal. Peanut planting media was maintained at high humidity for 7 days during inoculation process.

Evaluation of plant resistance to four races *S. rolfsii* infection was calculated based on symptom score, die plant percentage and plant growth. Symptom plant scoring was defined as: score 0 = no symptom, score 1= indicated necroses until 0.5 stem diameter, score 2= necroses from 0.5 - 0.75 stem diameter, score 3= totally necroses of stem diameter, score 4= similar score 3, stem and leaf appear wilt , and score 5= dead plant (Yusnita & Sudarsono 2004).

III. Results and Discussion

Characterization of the *S. rolfsii* races

Fungus *S. rolfsii* had been collected by 9 isolates from various centers of peanut production and host plant in Lombok Island, then characterized by the growth of colonies. The results of mycelia morphological observation could be seen in Table 2.

Table 2. Mycelia morphology of various races *S. rolfsii*

Isolate	Mycelia coloring	Mycelia Structure	Sclerotia size	Amount of day to form sclerotia
1	White	Thick rough	Big	14-20
2	White	Thick rough	Big	14-20
3	White	Thick rough	Big	14-20
4	White	Thick rough	Big	14-20
5	White	Thick rough	Big	14-20
6	White	Thick rough	Big	14-20
7	White	Thick rough	Big	14-20
8	White	Smooth thin	Small	4-6
9	White	Smooth thin	Big	10-14

Explanation :
Isolate 1 = peanut stem rot disease from region of North Lombok Tanjung Vilage 1
Isolate 2 = peanut stem rot disease from region of North Lombok Tanjung Vilage 2
Isolate 3 = peanut stem rot disease from region of North Lombok Tanjung Vilage 3
Isolate 4 = peanut stem rot disease from region of North Lombok Tanjung Vilage 4
Isolate 5 = peanut stem rot disease from region of West Lombok Labu Api Vilage 1
Isolate 6 = peanut stem rot disease from region of West Lombok Labu Api Vilage 2
Isolate 7 = peanut stem rot disease from region of North Lombok Akar-Akar Vilage
Isolate 8 = stem rot disease *to ornamental crop (*Amazon lily=*Eucharis glandiflora*)
Isolate 9 = stem rot disease *to ornamental crop* (*Neomarica longifolia*)

Table 2 showed that of the 9 isolates *S. rolfsii* collected, there were 4 isolates assumed different based on the characterization of mycelia morphology and them sclerotia, namely isolate 1, isolate 6, isolate 8, and isolate 9. The isolates difference were suspected because of the recombinant that occurs naturally in the field. Individual genotypes of fungal pathogens may differ in various characters, such as morphology, physiology and pathogenicity. Most of these characters are controlled by the genes present in the cell nucleus and some other characters are controlled by the cytoplasmic genes (Person and Ebba, 1975).

Characterization of *S. rolfsii* races was then performed by identification of MCG based on modification methods developed by Carling et al. (1994) and Bains & Bisht (1995). Nine isolates tested have been observed MCG. Figure 1 was a representation of MCG test among combinations of 3 isolates.

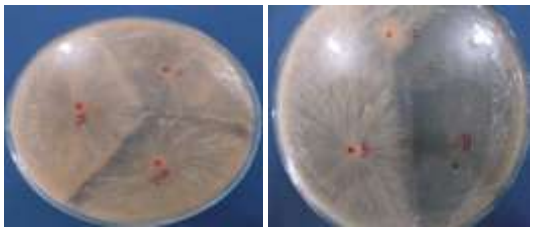

Figure 1. Example of MCG test results of various isolate combinations

Figure 1 showed that isolates 1 and 5 occur in compatibility whereas isolate 1 with isolate 9 did not have compatibility. Similarly, isolate 5 did not have compatibility with isolates 9. From the 9 isolates tested, there were only 4 isolates giving different results of MCG assay and they were considered to have a separate MCG, ie. isolates 1, 6, 8 and 9.

In vitro selection to identify resistant embryogenic callus to selective medium containing culture filtrate of various *S. rolfsii* races

Efforts to improve peanut plant resistance to stem rot disease from various races of S. *rolfsii* can be performed by in vitro selection on culture filtrate from various races of *S. rolfsii*. Embryogenic callus population that were generated from in vitro selection were reevaluated for its resistance to a selective medium containing culture filtrate from various races of *S. rolfsii*. Resistance of embryogenic callus population was determined by observing the embryogenic calluses response to 35% culture filtrate of various *S. rolfsii* races. In Table 3 and Table 4 showed that the percentage of living embryogenic callus number and SEs number per explant were determined by selective medium culture filtrate. Embryogenic callus derived from selected embryogenic callus in medium selective culture filtrate race-1 (MS-P16-FK-1) tended to be more resistant to culture filtrate race-1

(MS-P16-FK-1) and more susceptible to other selective medium culture filtrate. Selected embryogenic calluses on culture filtrate of S. *rolfsii* races 1, 6, 8, 9 were more resistant than embryogenic calluses that were selected on other culture filtrate media when re-evaluated on culture filtrate selection media containing races 1, 6, 8, 9. This occured because of the in vitro selection results caused in the accumulation of tolerant mutant cell/tissue to the culture filtrate *S. rolfsii*. Resistant variant cells/tissues during the selection period were proliferated on MS-P16 medium to obtained large amounts of embryogenic callus. According to Yusnita et al. (2005) that insensitive embryogenic callus to culture filtrate was able to perform the detoxification mechanism against oxalic acid released by *S. rolfsii*. Plant grape somatic embryos may be tolerant of selective media containing the culture filtrate of *Elsinoe ampelina* because of induction of detoxification enzymes during in vitro selection (Kulsova et al., 1997). The resistant embryogenic callus was then regenerated into planlet and tested for resistance in 4 races of *S. rolfsii* in subsequent experiments.

Table 3. Number of live explants (%) of various peanut embryogenic callus populations on culture filtrate media of *S. rolfsii*

Embryogenic callus populations (KE)	Number of live explants (%)			
	Media MS-P16-FK-1	Media MS-P16-FK-6	Media MS-P16-FK-8	Media MS-P16-FK-9
KE-Race-1	88,6	15,4	18,0	12,4
KE- Race-1-6	75,5	69,7	10,5	12,0
KE-Race-6	14,5	89,6	12,3	11,0
KE-Race-1-6-8	65,6	68,0	58,8	14,3
KE-Race-8	13,2	9,8	85,6	10,4
KE-Race-1-6-8-9	58,5	60,6	60,0	57,6
KE-Race-9	14,5	12,0	12,4	87,8
KE (control=without in vitro selection)	3,5	4,5	2,4	3,0

Explanation:
KE-Race-1= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 1
KE-Race-1-6= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 1 & 6
KE-Race-6= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 6
KE-Race-1-6-8 = embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 1,6 & 8
KE-Race-8= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 8
KE-Race-1-6-8-9= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 1,6,8 &9
KE-Race-9 = embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 9
Media MS-P16-FK-1= selective media containing culture filtrate *of S. rolfsii* race 1
Media MS-P16-FK-6= selective media containing culture filtrate *of S. rolfsii* race 6
Media MS-P16-FK-8= selective media containing culture filtrate *of S. rolfsii* race 8
Media MS-P16-FK-9= selective media containing culture filtrate *of S. rolfsii* race 9

Table 4. Number of ES per explant from various population of embryogenic callus on culture filtrate medium of *S. rolfsii*

Embryogenic callus populations (KE)	Number of ES per explant			
	Media MS-P16-FK-1	Media MS-P16-FK-6	Media MS-P16-FK-8	Media MS-P16-FK-9
KE-Ras-1	12,8	2,3	3,0	4,3
KE-Ras-1-6	8,4	8,2	2,4	3,0
KE-Ras-6	2,0	13,5	3,0	2,5
KE-Ras-1-6-8	7,5	7,0	6,4	2,0
KE-Ras-8	3,2	3,0	14,2	3,2
KE-Ras-1-6-8-9	6,7	7,5	7,5	8,0
KE-Ras-9	3,2	2,5	2,5	14,0
KE (control=without in vitro selection)	2,0	2,2	2,2	2,6

Explanation:

KE-Race-1= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 1
KE-Race-1-6= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 1 & 6
KE-Race-6= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 6
KE-Race-1-6-8 = embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 1,6 & 8
KE-Race-8= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 8
KE-Race-1-6-8-9= embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 1,6,8 &9
KE-Race-9 = embryogenic callus population that were generated from in vitro selection on culture filtrate media *S. rolfsii* race 9
Media MS-P16-FK-1= selective media containing culture filtrate *of S. rolfsii* race 1
Media MS-P16-FK-6= selective media containing culture filtrate *of S. rolfsii* race 6
Media MS-P16-FK-8= selective media containing culture filtrate *of S. rolfsii* race 8
Media MS-P16-FK-9= selective media containing culture filtrate *of S. rolfsii* race 9

There was also a tendency that embryogenic calluses selected in selective media more than one race resulted fewer percentage number of live embryogenic callus and ESs per explant than embryogenic calluses selected in only one-race culture filtrate medium. This proved that the mutants had genetic character as polygenic with the role of this gene was minor gene compared the other mutants having genetic character as monogenic with the role of this gene was major genes (Robinson, 1987).

Evaluation of peanut somaclons against infections of various races of *S. rolfsii* fungus in Greenhouse

Infections of *S. rolfsii* fungal cause dead plants before pods producing. Table 5 showed that peanut crops regenerated from selected ESs in various races cultures filtrate resulted in fewer percentage of dead plants than standard plants (peanut line without in vitro selection). Plants regenerated from in vitro selection ES on culture filtrate more than one race (races 1, 6, 8, and 9) resulted in a higher percentage of dead plants when compared that selected in culture filtrat one race or two races or three races. In selected embryogenic calluses on culture filtrate various races 1, 6, 8, and 9 resulted more dead peanut plants when infected with various races of pathogens (1, 6, 8 and 9) ie 32.2%, 32.3 %, 27.8%, and 37.3% respectively (Table 5).

Table 6 showed that all the somaclones and standard plants (plants without in vitro selection) were infected with *S. rolfsii* with a symptom score of 1.3 to 4.6. High score experienced by standard plants. All selected somaclone plants had lower symptom scores than standard plants. Somaclone plants regenerated from in vitro selection ES on culture filtrate on more than one race (races 1, 6, 8, and 9) yielded greater symptom scores when compared to if selected in one race or at two races or at the three races. The lowest disease symptom scores were found in somaclone plant selected on culture filtrate races 1-6 and the somaclones plant were infeted by races 1 and 6. These somaclones were specifically resistant to race 1 and race 6 and were not resistant to other races.

Table 5. Percentage of dead somaclone plants after inoculated with *S. rolfsii* at the R1 generation in greenhouse

Somaclones Plant Population	Races *S. rolfsii*			
	1 *)	6 *)	8 *)	9 *)
KE-Race-1	25,8 aA	36,2 bC	36,3 bC	37,2 bC
KE-Race-1-6	28,0 aAB	28,9 aB	37,5 bC	38,1 bC
KE-Race-6	32,6 bB	22,5 aA	41,7 cD	37,4 cC
KE-Race-1-6-8	21,7 aA	27,5 bB	25,6 bAB	37,3 cC
KE-Race-8	38,3 cC	40,1 cD	20,0 aA	32,3 bB
KE-Race-1-6-8-9	32,2 bB	32,3 bBC	27,8 aB	37,3 cC
KE-Race-9	38,6 cC	39,2 cD	33,4 bC	20,0 aA
Control plant =without in vitro selection)	49,1 aD	43,1 aD	46,8 aD	48,5 aD

Explanation:
KE-Race-1= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1
KE-Race-1-6= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1 & 6
KE-Race-6= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 6

KE-Race-1-6-8 = plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1,6 & 8
KE-Race-8= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 8
KE-Race-1-6-8-9=plants resulted from the selection of embryogenic callus on culture filtrate media *S.rolfsii* race 1,6,8 &9
KE-Race-9 = plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 9
*) The number followed by the same small letters at the same row and followed by the same capital letters at same colum were not significaltly different at 5% level by Duncan test.

Table 6. Disease symptom scores of R1 generation peanut socalones after inoculation with various races *S. rolfsii* in greenhouse

Somaclones Plant Population	Races of *S. rolfsii*			
	1 *)	6 *)	8 *)	9 *)
KE-Race-1	2,0 aA	2,9 bB	2,2 aA	2,8 bB
KE-Race-1-6	1,8 aA	1,6 aA	2,6 bA	2,2 abA
KE-Race-6	2,7 bB	2,0 aA	3,7 cB	3,3 cC
KE-Race-1-6-8	2,3 aA	2,3 aB	2,6 aA	3,4 bC
KE-Race-8	3,3 bB	2,7 aB	2,3 aA	3,4 bC
KE-Race-1-6-8-9	2,1 aA	2,6 bB	2,3 aA	2,7 bB
KE-Race-9	3,2 cB	2,5 bB	2,5 bA	1,8 aA
Control plant =without in vitro selection)	3,8 aC	3,8 aC	3,9 aB	3,7 aC

Explanation:
KE-Race-1= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1
KE-Race-1-6= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1 & 6
KE-Race-6= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 6
KE-Race-1-6-8 = plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1,6 & 8
KE-Race-8= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 8
KE-Race-1-6-8-9=plants resulted from the selection of embryogenic callus on culture filtrate media *S.rolfsii* race 1,6,8 &9
KE-Race-9 = plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 9
Symptom score: score 0 = no symptom, score 1= indicated necroses until 0.5 stem diameter, score 2= necroses from 0.5-0.75 stem diameter, score 3= totally necroses of stem diameter, score 4= similar score 3, stem and leaf appear wilt , and score 5= dead plant
*) The number followed by the same small letters at the same row and followed by the same capital letters at same colum were not significaltly different at 5% level by Duncan test.

The effectiveness of pathogenicity of *S. rolfsii* fungi is closely related to its ability to produce oxalic acid. The secretion of the oxalic acid toxin from *S. rolfsii* is toxic to the host plant in order to kill the tissue of the host plant. The experiment result of Ferrar and Walker (1993) that *S. rolfsii* could produce milimolar oxalate concentration in infected tissue. This oxalic acid could inhibit stomatal closure so that the plant will undergo excessive transpiration so that the plants experience drought (wilting) (Kolkman and Kelly, 2000). Oxalic acid released could increase the virulence of *S. rolfsii* fungi. This oxalic acid compound could also cause chlorosis and leaf necrosis during the first stadia of plant development (Backman & Brenneman 1997; Cessna et al. 2000). Table 7 explained the ability of several races of *S. rolfsii* to accumulate oxalic acid in peanut plant tissues. Each *S. rolfsii* race accumulated oxalic acid of different amounts in each peanut genotype. Race 6 and 9 accumulated more oxalate in the Kancil peanut genotype, while race 1 accumulates the most oxalic acid in the peanut G300 line. This showed that each *S. rolfsii* races had a different reaction to peanut genotypes.

Table 7. Oxalic acid content in peanut tissue infected by various Race *S. rolfsii*

Peanut Cultivar	Oxalic acid content of *S. rolfsii races (mg)*			
	1	6	8	9
Kancil	0,28 b *)	1,30 a	0,38 b	1,40 a
G300	0,63 a	0,32 b	0,22 c	0,27 c

*) The numbers followed by the letters on the same row are not significantly different at Duncan test 5%

Dry weight of pods (g) produced by peanut somaclones grown in Greenhouse

Infections of *S. rolfsii* cause economic losses to peanut crops. This pathogen attack causes necroses light brown to dark brown in stems, branches and ginofor. Further infections in the stems and branches of the

plant cause wilted plant, leaves fall out and dead plants (Melouk & Backman 1995). Pathogen control strategy is very important to inhibit the progression of the disease. The use of resistant cultivars from in vitro selection is a promising alternative to increasing peanut production.

The experiment results showed that peanut somaclone inoculated with *S. rolfsii* fungi tended to give many pods and more resistant to *S. rolfsii* attack than plants that did not pass the in vitro selection (standard plants). Peanut plant generated from in vitro selection also resulted the percentage of dead plants due to infection of *S. rolfsii* lower than the standard plants. Among peanut somaclones evaluated, peanut somaclone selected in the culture filtrate race 8 produced the highest dry pods weight per plant. Susceptible peanut plants to disease cause more damage. Resistant plants are able to combat the attack of the fungus through a defense mechanism already possessed by plants (Hammond-Kosack & Jones 1996).

Peanut somaclones infected by various *S. rolfsii* races produced different dry pod weight. Peanut somaclones regenerated from in vitro selection ES on culture filtrate on more than one race (races 1, 6, 8, and 9) tended to result in lower dry pod weight than if selected in one race or at two races or at three races. The selection pressure experienced by the embryogenic calluses caused the resistance experienced by the somaclones to be lower so that the pods were damaged and the weight of the dry pod becomes lower (Table 8).

Table 8. Dry pods weight(g) per plant of R1 generation peanut somaclones after infected by various *S. rolfsii* races in greenhouses

Somaclones Population	Races of *S. rolfsii*			
	1 *)	6 *)	8 *)	9 *)
KE-Race-1	13,7 aA	10,9 bC	10,9 bCD	9,9 cC
KE- Race -1-6	13,4 aA	15,4 aA	11,4 bC	12,9 abA
KE- Race -6	11,6 bB	14,2 aA	12,7 bB	14,0 aA
KE- Race -1-6-8	10,0 bBC	13,4 aB	14,7 aA	12,7 abA
KE- Race -8	11,6 bB	15,0 aA	15,9 aA	11,4 bB
KE- Race -1-6-8-9	13,0 aA	13,0 aB	12,5 bB	13,0 abA
KE- Race -9	11,4 bB	11,6 bC	11,3 bC	13,8 aA
Control plant = without in vitro selection)	9,6 aC	11,2 aC	10,2 aD	9,5 aC

Explanation:
KE-Race-1= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1
KE-Race-1-6= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1 & 6
KE-Race-6= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 6
KE-Race-1-6-8 = plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 1,6 & 8
KE-Race-8= plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 8
KE-Race-1-6-8-9=plants resulted from the selection of embryogenic callus on culture filtrate media *S.rolfsii* race 1,6,8 &9
KE-Race-9 = plants resulted from the selection of embryogenic callus on culture filtrate media *S. rolfsii* race 9
*) The number followed by the same small letters at the same row and followed by the same capital letters at same colum were not significaltly different at 5% level by Duncan test.

IV. Conclusions

1. Embryogenic callus selected in a selection medium containing culture filtrate more than one *S. rolfsii* race generated a percentage of the number of live ESs and the number of ES per explant fewer than embryogenic callus that was selected in culture filtrate media containing one race. Overall, the embryogenic callus selected on selective media containing culture filtrate more than one race *S. rolfsii* fungus was more resistant to selective media containing culture filtrate many races than the embryogenic callus which was selected only on one race *S. rolfsii*.
2. Peanut somaclones produced from embryogenic callus selection on media containing culture filtrate various races of *S. rolfsii* had different responses to several races of *S. rolfsii* (races 1, 6, 8, and 9) infection. The somaclone population derived from embryogenic callus selected in the selection medium containing more than one race culture filtrate resulted in plant damage due to *S. rolfsii* infection greater than the peanut somaclones derived from embryogenic callus which were selected only on one race culture filtrate medium. Overall, somaclones derived from embryogenic calli were selected on selective media containing culture filtrate more than one race the *S. rolfsii* fungi were more resistant from infection of various *S. rolfsii* races compared with somaclones derived from embryogenic callus that were selected only on selective medium containing culture filtrate of one race *S. rolfsii.*

3. Infection of various *S. rolfsii* races produced different dry pods weight on somaclones peanut planted in a greenhouse. The somaclone peanut plant regenerated from the selection of embryogenic callus on selective media containing culture filtrate more than one race (races 1, 6, 8, and 9) resulted a lighter weight of dry pods compared with somaclones peanut produced from the selection of embryogenic callus on selective media containing culture filtrate one race, two races or three races *S. rolfsii*. The somaclone peanut plant regenerated from the selection of embryogenic callus in culture selective filtrate media resulted in heavier dry pod weight than the control plants (without in vitro selection) in greenhouse plantings.

Acknowledgment

This research was conducted at the expense of the Directorate General of Higher Education of the Ministry of Research, Technology and Higher Education of Indonesia through the Research Scheme of Unram's Decentralized Research Fund for Fiscal Year 2015.

References

[1] Backman PA, Brenneman TB. 1997. Stem-rot. In : Burelle NK, Porter DM, Kabana RR, Smith DH, Subrahmanyam P, (Ed.). Compedium of peanut disease. American Phytopathological Society, St. Paul, MN.
[2] Bains PS., Bisht VS. 1995. Anastomosis group identity and virulence of Rhizoctonia solani isolates collected from potato plants in Alberta, Canada. Plant Dis. 79 : 241-242
[3] Benhamou N, Chert I. 1996. Parasitism of *Sclerotium rolfsii* by *Tricoderma harzianum* : ultrastructural and cytochemical aspects of the interaction. Phytppathology 86:405–416.
[4] Carling DE, Rothrock CS, MacNish GC, Sweetingham MW, Brainard KA, Winters SW. 1994. Characterization of anastomosis group 11 (AG-11) of Rhizoctonia solani. Ecology and Epidemiology 84: 1357-1393.
[5] Cazares, B.X., Ortega F.A.R., Elenes L.F., Medrano R.R. 2011. Drought tolerant in crop plant. American Journal of Plant Physiology. Academic Journal Inc.
[6] Cessna SG, Sears VE, Dickman MB, Low PS. 2000. Oxalic acid, a pathogenesis factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. The Plant Cell 12:2191-2199.
[7] Ferrar PH and Walker JRL. 1993. o-Diphenol oxidase inhibition—an additional role for oxalic acid in the phytopathogenic arsenal of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii.* Physiol Mol Plant Pathol 43: 415–422.
[8] Gamborg OL, Miller RA, Ojima K. 1968. Nutrient requirement of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158.
[9] Hammond-Kosack KE, Jones JDG. 1996. Resistance gene-dependent plant defence responses. Plant Cell 8:1773 - 1791.
[10] Hemon AF, Ujianto L, Sudarsono. 2006. Seleksi berulang dan identifikasi embrio somatik kacang tanah yang insensitif polietilena glikol (PEG) dan filtrat kultur *Sclerotium rolfsii*. Agroteksos 16:21-32.
[11] Hemon A.F. 2009. Penggunaan variasi somaklonal dan seleksi in vitro untuk mendapatkan plasma nutfah tanaman resisten terhadap penyakit. Seminar Nasional dan Pameran Hasil-hasil Penelitian (Dalam rangka Dies Natalis Universitas Mataram ke 47). Lembaga Penelitian Universitas Mataram, Mataram 29-30 September 2009.
[12] Hemon A.F, Sumarjan, Ernawati L. 2017. Perbaikan karakter melalui seleksi *in vitro* untuk mendapatkan kacang tanah cv. Lokal Bima *double characters* : toleran cekaman kekeringan dan resisten terhadap infeksi berbagai *race* cendawan *Sclerotium rolfsii*. Laporan Penelitian Universitas Mataram.
[13] Jain SM. 2001. Tissue culture-derived variation in crop improvement. Euphytica 118 :153-156.
[14] Karp A. 1995**.** Somaclonal variation as a tool for crop improvement. Kluwer Acad. Publ. The Netherlands.
[15] Kolkman JM and Kelly JD. 2000. An indirect test using oxalate to determine physiological resistance to white mold in common bean. Crop Sci 40: 281–285
[16] Kuksova VB, Piven NM, Gleba YY. 1997. Somaclonal variation and in vitro induced mutagenesis in grapevine. Plant Cell Tiss. Org. Cult. 49:17–27.
[17] Matsumoto K, Barbosa ML, Souza LAC, Teixeira JB. 1995. Race I fusarium wilt tolerance on banana plants selected by fusaric acid. Euphytica 84:67–71.
[18] Melouk HA and Backman PA. 1995. Management of soil-borne fungal pathogens. Di dalam : Melouk HA, Shokes FM. (Ed.). Peanut healt management. APS Press St. Paul. MN
[19] Murashige T, Skoog F. 1962. A revised media for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant 15:473-493.
[20] Person, C and Ebba, T. 1975. Genetics of fungal pathogens. *Genetics, Suppl* 79, 397–408.
[21] Porter DM, Smith DH, Rodriguez-Kabana R. 1982. Peanut plant disease.In: Patee HT, Young CT (ed). *Peanut Science and Technology.Yoakum*, TX: American Peanut Research and Education Society.p 348-354.
[22] Punja ZK. 1985. The biology, ecology, and control of *Sclerotia rolf*sii. Ann. Rev. Phytopathol. 23:124–135.
[23] Robinson, R.A. 1987. Host management in crop pathosystems. Macmillan Publishing Company, New York.262 p.
[24] Yusnita, Sudarsono. 2004. Metode inokulasi dan respons ketahanan 30 genotipe kacang tanah terhadap penyakit busuk batang akibat infeksi *Sclerotium rolfsii Sacc.* Hayati 11:53-58.
[25] Yusnita, Widodo, Sudarsono. 2005. In vitro selection of peanut somatic embryos on mediun containing cultur filtrate of *Sclerotium rolfsii* and plantlet regeneration. Hayati 12:50-56.

A. Farid Hemon In Vitro Selection Of Embryogenic Callus To Produce Resitant Peanut Plant Against On Various Races Of Sclerotium Rolfsii In Lombok Island." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), vol. 10, no. 10, 2017, pp. 43-51