Peanut Mottle Virus (Ptmtv) Contamination on Peanut Seeds Collected from Several Locations and Its Elimination by Hot Water Treatment

Pudji Sulaksono

Laboratory of Plant Pests And Diseases Department, Agricultural Faculty,
Tadulako University, Palu 2006
pudsul@yahoo.co.uk

Abstract

The following observations on the seemingly prevalent occurrence of peanut (Arachis hypogaea) *seed contaminations by PtMtV virus in the surrounding area of Palu, Central Sulawesi were conducted to determine the level of seed contamination and whether hot water treatment can be used to eliminate PtMtV virus from the seeds. Peanut seeds were collected from a number of locations and after soaking them in unheated water (29°C) and heated water (55, 60 and 65°C) for 10 minutes they were allowed to germinate, then transplanted and grown in pots in screen house. The effects of hot treatments on seed germinability, leaf formation, frequency of infection on surviving plants, and plant biomass production were determined. Seeds soaked in heated water showed different germinability, grew to become plants with different leaf formation and plant biomass production. Heat treatments gave different frequencies of infection on the plants. However, not a single heat treatment gave satisfactorily results in terms of giving zero or low infection and at the same time giving desirable seed germinability, leaf number, and plant biomass. The prospect of using hot water treatment at 55°C or lower with longer soaking duration as a method for PtMtV virus elimination from peanut seeds is discussed.*

Key words*: virus, PtMtV, peanut,* Arachis hypogaea*, soaking, heat treatment, mottle*

Introduction

Peanut (*Arachis hypogaea*) growing regions in Central Sulawesi Province include Tavaeli, Sirenja, Banggai Islands, and Parigi. Peanut was also grown in vast numbers in the region of Morowali, in east-southern part of the province. Peanut Mottle Potyvirus (PtMtV) disease has been recovered from peanut seeds collected from a number of peanut growing areas around Palu, Central Sulawesi (Nurrahmah, 2004). In 2003, the present author personally observed an almost 100% peanut mottle virus incidence in plants grown from several seed lots of peanuts bought from farmers who normally used the same seeds for planting their crops.

PtMtV occurs in all peanut growing countries, causing yield reduction reaching up to 40%, and being considered of global economic importance (Šutić *et al.*, 1999). In Indonesia, the disease has been known to occur in Java, Kalimantan, Sulawesi and Sumatra (Murayama *et al.*, 1998). Although the disease does not reduce seed weight, seed germinability, or seed size, in Central Java, it has reported to cause peanut yield reduction in the range between 3 and 29% (Semangun, 1993). Five to six percent peanut yield losses have been recorded in the field with the occurrence of 26% infection (Damicone and Sherwood, 2003). Peanut kernel dry weight and pod dry weight losses due to the PtMtV in a glasshouse experiment are approximately as much as 30 and 28%, respectively, with the plants are given recommended rate of fertilization (Sulaksono, 2005). Yield losses caused by virus infection can be variable from season to season seemingly depending on many factors. This has been exemplified with Bean Pod Mottle Virus (BPMV) infection on soybean. Cihlar-Strunk and Langham (2004) investigated effect of BPMC infection on soybean yield for two years. They reported that infections of BPMV on ten lines of soybean result in yield losses ranging from 1% to 19% in 2002 and 16% to 56% in 2003. This finding suggests that little losses caused by a viral disease observed in

one occasion may mean big losses in other occasion, depending on a number of uncontrollable factors. It is then fair to suspect that PtMtV may cause peanut yield losses higher than the figures already reported when favorable conditions for disease are prevailing.

Economic losses due to viral disease can also be in the form of reduced or spoiled seed quality. BPMV disease causes poor soybean seed quality and reduce marketable price (Ziems *et al.*, 2001). There is also other kind of loss. PtMtV-infected seeds of peanut do not change in appearance. But since the virus is carried through the seeds therefore germplasm exchange by means of seeds risks virus disease transmission to formerly virus-free area. Contamination of previously virus-free area with virus can bring damaging consequences to the plant protection conditions in the area concerned. The disease is also known to be spread by several species of vector insects, generally those of Aphididae family members.

The situation with peanut and PtMtV disease in Palu can be regarded as frustrating. Peanut seed contamination most likely occurs in high incidence while control of the PtMtV disease is dependent more on virus-free certified seeds, resistant varieties and control of virus vectors. Certified seeds and resistant cultivars are not yet available or will not likely be available within several years in regions around Palu. Chemical control of Aphid insect vectors can be done but its effectiveness for curbing virus spread is questionable if seed contamination is already high. It is indispensably urgent to verify the degree of contamination in local peanut seeds to work out the possibility of using the seeds for planting material. It is also essential to evaluate any practical and relatively cheap control methods. Heat treatment for plant propagation material has not been being in much discussion in the recent years. Quite a number of years ago it had been generally referred by workers as a way for virus inactivation or elimination from planting material (Johnstone and Wade, 1974; Nair, 1973; Sward and Hallam, 1976; Kriedemann *et al*., 1976; Hernadi, 1996; Smee and Ikin, 1975). Heat treatment method that is used to eliminate the virus in seeds should be one that does not detrimentally affect growth and yield of the crops grown from the seeds. Heat treatment has been used by Hernadi (1996) successfully to reduce seed infection of groundnut mosaic virus from 35 to 5% without seemingly being deleterious to the plant growth and development. Since seed heat treatment is cheap and practicable even at rural setting this method may offer solution for the problem of PtMtV disease in peanut.

This paper reports the investigation for determining the incidence of PtMtV contamination of peanut seeds and heat treatment temperature that eliminates viral infection from seeds while keeping growth and development quality of the seeds.

Material and Method

The experimental work was conducted in the screen house of Plant Pests and Diseases Department of Tadulako University from September to November 2004. Seeds of peanut were collected from five peanut planting locations situated in three Districts of Donggala Regency. Those locations were Kawarana and Solove (Dolo District), Sidera and Lolu (Biromaru District), and Guntarano (Tavaeli District). The seeds were purchased in their shells from the grower. After being removed from their shells the kernels (seeds) of each location were put in a container. A glass containing water in an amount enough to soak 25 seeds was heated in a water bath fixed with a thermostat. By using a rod thermometer, the temperature of the water in the container was checked regularly and when it achieved 55ºC the thermostat was fixed. The seeds were put in the hot water in the container held in the water bath for ten minutes and after that were removed from the water quickly and laid on a clean table. At any repetition checking was made to ensure that the water temperature was correct. These steps were repeated for seeds from every location. The whole steps were then repeated with temperature of 60 and 65ºC. To be used for control, seeds from each location were soaked in water having room temperature

for ten minutes. The room temperature of this water was found to be 29ºC. Three replicates were made for every treatment.

The treated seeds were planted in a tray already filled soil and allowed to grow in the laboratory (a tray was planted with 25 seeds from the same location of origin and of the same heat treatment). Observation was made for the number of seeds that developed to normal and abnormal seedlings and that showed symptom of PtMtV infection. After two weeks, the surviving plants were transferred to polybag and moved to screenhouse.

Fertilizers at the dosages equivalent to the recommended ones (0.075g urea, 0.15g SP36, and 0.075g KCl per polybag) were given to the plant when the plants were 15 days old (a day after transplantation). The fertilizers were placed at and mixed with the soil at about 5 cm away from the stem base. Watering was given twice every day, in the morning and at the dusk time. The following variables were measured:

a. Germinability (in percent) and vigor of the seeds in the trays. Germinability was determined by dividing the number of normally germinating seeds by the total number of seeds observed. Vigor status was established by the physical appearance of the seed growth.
b. Incubation period of PtMtV infection symptom on the leaves was determined with plants still on the tray.
c. After the plants were moved to the screenhouse disease incidence was determined at 2, 3, 4, 5 dan 6 weeks after seeding. Percent value was calculated by dividing the number of plants with disease symptom with the total number of surviving plants observed.
d. Number of leaves per plant was determined from three plants taken randomly from the existing surviving population at 2, 3, 4, 5 and 6 weeks after seeding.
e. Dry weight of whole individual plant. This was done by removing the plant with its roots, removing the soil from the root by washing, and placing the plant material in the oven until constant weight. The dried plant was then placed on a balance to weigh.

Result and Discussion

Level of seed contamination by PtMtV can be indicated by the frequency of infection given by hot water treatment at 29°C (control) (Figure 1) because this temperature was the unheated water temperature used in the experiment. Observation on week 4 obtained approximately 20 % of seeds contaminated by the virus given by this level of temperature. This figure is much lower than the result of former observation of the present author in 2003 (namely nearly 100%) while higher than those informed by Brunt *et al.* (1996) and comparable to those stated by Murayama *et al.* (1998). Seed contamination level of 20% is very significant epidemiologically for this represents the presence of large initial population of disease. The presence of seed contamination even at low levels could be important for virus perpetuation and dissemination and could provide inoculums source that may have a considerable impact on crop production. This kind of situation has been pointed out by Yang *et al.* (1997) to have been found with barley stripe mosaic horde virus, pea seed-borne mosaic potyvirus, lettuce mosaic potyvirus, tomato black ring nepovirus, and raspberry ring spot nepovirus. If many cultivars of peanuts grown in Central Sulawesi are susceptible to PtMtV and the insect vectors (*Aphis craccivora, A. gossypii, Hyperomyzus lactucae, Myzus persicae, Rhopalosiphum padi*) are also present in sufficient numbers in the field then peanut cultivation in this area will be seriously threatened by PtMtV.

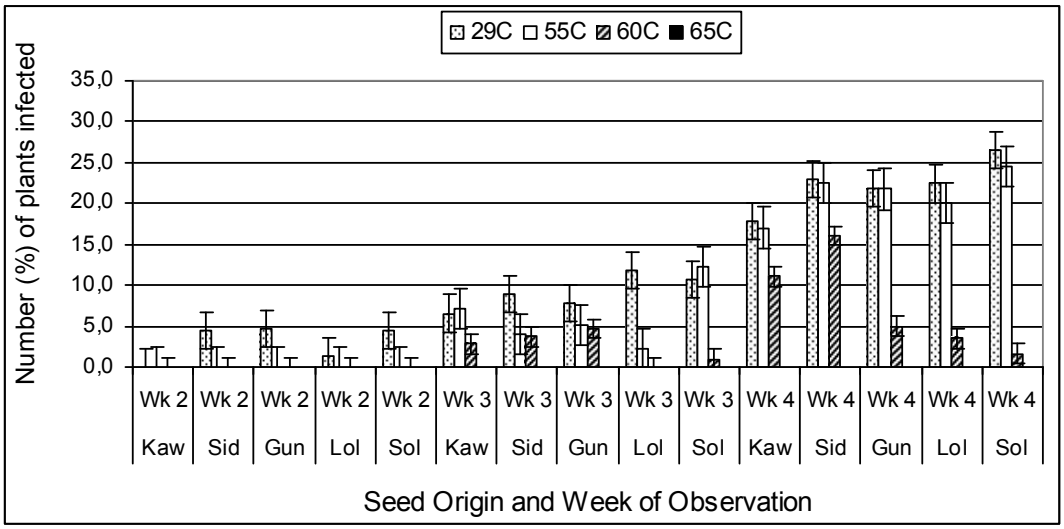

Figure 1. PtMtV infection frequency on peanut plants grown from seeds previously treated with hot water (vertical bars represent the standard errors). Note: No symptoms could be detected visually on the plants on weeks five and six and observation for symptoms was then terminated.

Hot water treatments affect seed infection levels, seed germinability, leaf formation and plant biomass production irrespective of the origins of the seeds used (Figures 1 to 4). Germinability was significantly reduced after the seeds were soaked in water at 60 and 65°C but was relatively not changed after being soaked in water at 55°C as compared to germinability given by soaking at 29°C (control). Although soaking at 60°C results in significantly low level of infection and gives leaf formation comparable to those given by soaking at 29°C (control) and at 55°C but this achievement is gained at the expense of great loss of seed germinability and total plant biomass. Soaking at 65°C was found even to result in very poor seed germinability and very poor leaf formation and production of plant biomass. From overall results (Figures 1 to 4), none of the hot water treatments studied seems to be a satisfactory method for freeing seed from PtMtV infestation.

The PtMTV virus's thermal inactivation point (TIP) is approximately between 53 and 65°C (Murayama *et al*., 1998; Brunt *et al*., 1996). Therefore, soaking seeds in water at 55°C should be adequate for at least inactivating the virus, although this was not the case in the present experiment. Exposure of seeds for 10 minutes to the heat in this experiment might be too short to allow the temperature of 55°C to inactivate or kill the virus; instead a longer exposure period may be required. A sustained period of 3-6 month-exposure to temperature of 37-40°C for grapevine plant material is needed to eliminate virus from the material (Kriedemann *et al*., 1976). To aid the survival of the plant material in the rigorous conditions due to heating, the workers gave the material CO_2 enrichment treatment after exposure to the heat. In connection with this it is speculated that the hot water treatment of 55°C can probably be used for eliminating PtMtV from peanut seeds provided sufficient time of exposure is given. For this purpose, future work should be directed at determining appropriate soaking duration of seeds in heated water at 55°C. The method of enriching the heated seeds with CO_2 for seed survival may also need to be evaluated.

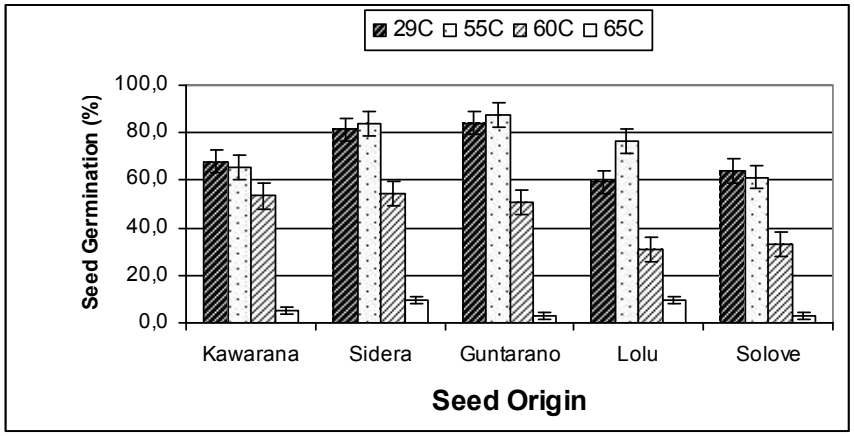

Figure 2. Germinability of peanut seeds taken from different places of origin and treated with hot water at various temperatures (vertical bars represent the standard errors)

Sward and Hallam (1976) investigated structural changes in the meristem tips of potato following heat therapy and observed abberant changes in endoplasmic reticulum, ribosomes, mitochondria, chloroplasts and membrane systems. They stated that a combination of these changes retard viral spread and development in the heated meristem. Rashid *et al.* (2004) observed more than 70% of the non-primed mungbean plants (*Vigna radiata*) to have severe or lethal symptoms of mungbean yellow mosaic virus (MYMV) whereas only 14% of the primed plants were similarly affected. The investigators conducted the seed priming with water for 8 hours. The marked differences between priming treatments in the incidence of disease were also found to reflect in the components of yield. Primed crops produced 80% more above-ground biomass (3.3 versus 1.9 t ha ^{1}), 264% more pod yield (1.0 versus 0.28 t ha ^{1}) and 415% more grain (0.36 versus 0.07 t ha ^{1}) than did non-primed crops. Thus in the light of these findings and the report of Kriedemann *et al.* (1976) mentioned earlier that grapevine plant may be freed from virus with heat therapy at 37-40°C it may be possible to obtain a temperature lower than 55°C administered for certain duration that can be used for PtMtV elimination from peanut seeds and at the same time gaining well growing and yielding crops.

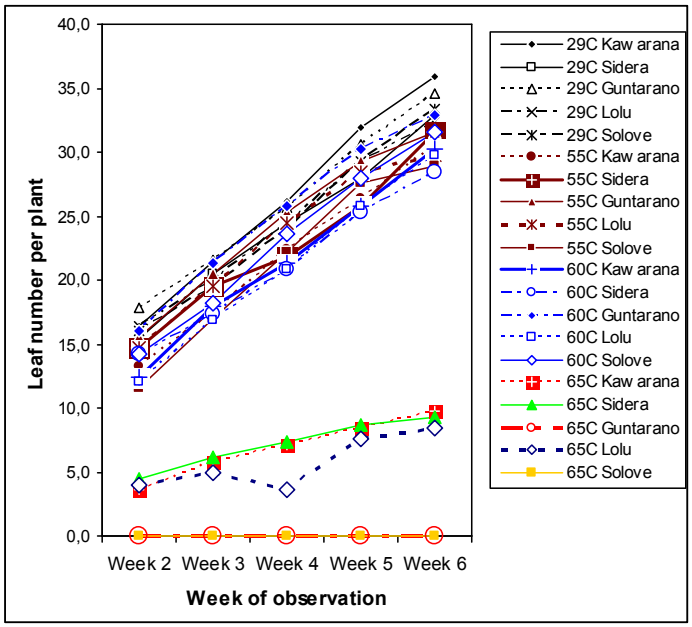

Figure 3. Effect of soaking peanut seeds gained from different origins in heated water on the formation of leaf

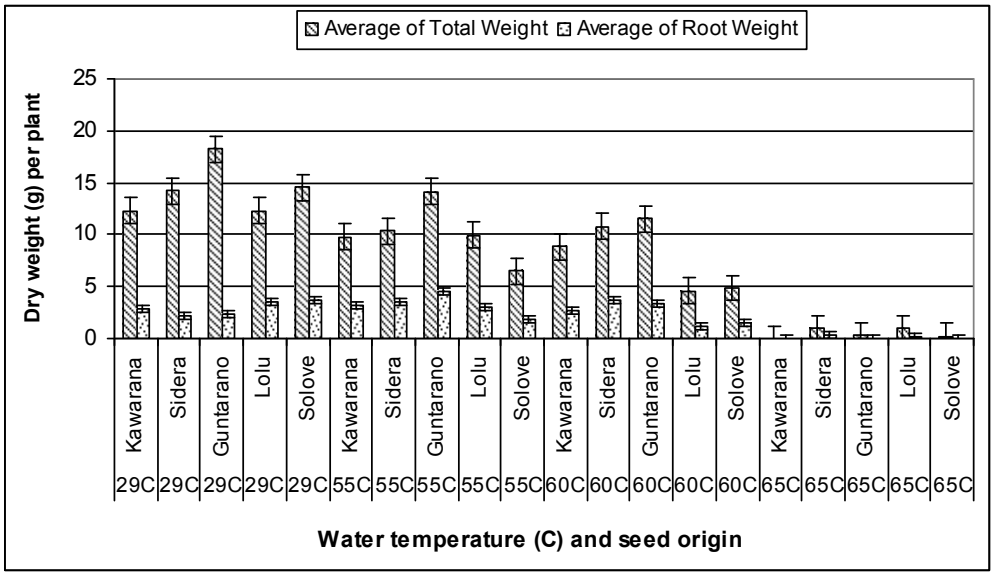

Figure 4. Effect of soaking peanut seeds gained from different origins in heated water on the plant biomass (vertical bars represent the standard errors)

Conclusion

The level of PtMtV contamination of peanut seeds used for planting crops in regions around Palu, Central Sulawesi, is about 20% and this is considered epidemiologically alarming. Among the hot water treatments studied using temperatures of 29, 55, 60, and 65°C, none is satisfactory to be used for elimination method of PtMtV from peanut seeds. Duration of soaking for 10 minutes is thought to be too short for hot water therapy at 55°C to be effective. There is a need for further work to investigate whether water temperature of 55°C or lower combined with soaking durations longer than 10 minutes can be effective for both eliminating PtMtV from peanut seeds and retaining well growing and productive crops.

Acknowledgement

The present author is deeply indebted to Roosmiaty Mangantjo for helping in the carrying out and contributing to the funding of the experiments.

References

Brunt, A.A., K. Crabtree, M.J. Dallwitz, A.J. Gibbs, L. Watson, and E.J. Zurcher (eds.) (1996 onwards). `Plant Viruses Online: Descriptions and lists from the VIDE database. Version: 20th August 1996.' URL http://biology.anu.edu.au/Groups /MES/vide/ (accessed 20th August 1996).

Cihlar-Strunk C. L. and M.A.C. Langham. 2004. Bean pod mottle virus effects on yield of ten soybean lines**.** APS Abstracts of Presentations. Phytopathology 94: S19.

Damicone, J.P. and J.L. Sherwood. 2003. Viral diseases of peanut (on line). Available online at http://www.pearl.agcomm.okstate.edu/plant diseases/f.7187.pdf (accessed 14th August 2003).

Hernadi, R., 1996. Pengaruh perlakuan ar panas terhadap biji kacang tanah pembawa groundnut mosaic virus. Buletin Budidaya Pertanian 2(1): 17-25.

Johnstone, G.R. and G.C. Wade. 1974. Therapy of virus-infected plants by heat treatment ii. Host protein synthesis and multiplication of tomato Aspermy virus at 36°C. Australian Journal of Botany 22(3): 451 – 460 (Abstract).

Kriedemann, P.E., R.J. Sward, and W.J.S. Downton. 1976. Vine response to carbon dioxide enrichment during heat therapy. Australian Journal of Plant Physiology 3(5): 605-618 (Abstract).

Murayama, D., H.O. Agrawal, T. Inoue, J. Kimura, E. Shikata, K. Tomaru, T. Tsuchizaki, and Triharso (eds.). 1998. Plant Viruses in Asia. Gadjah Mada University Press, Yogyakarta.

Nair, N.G. 1973. Heat therapy of virus-infected cultures of the cultivated mushroom *Agaricus bisporus*. Australian Journal of Agricultural Research 24 (4): 553 – 541 (Abstract).

Nurrahmah, A., 2004. Keberadaan Penyakit Virus Terbawa Benih pada Kacang Tanah yang Dipasarkan di Daerah Biromaru. Skripsi, Fakultas Pertanian, Universitas Tadulako.

Rashid, A., D. Harris, P. Hollington, and S. Ali. 2004. On-farm seed priming reduces yield losses of mungbean (*Vigna radiata*) associated with mungbean yellow mosaic virus in the North West Frontier Province of Pakistan. Crop Protection 23 (11): 1119-1124 (Abstract).

Semangun, H. 1993. Penyakit-penyakit Tanaman Pangan di Indonesia. Gadjah Mada University Press, Yogyakarta.

Smee, L and R. Ikin. 1975. Technical notes. A simple low-cost cabinet for heat therapy of virus infected plants. Australian Plant Pathology Society Newsletter 4 (2): 15 – 16.

Sulaksono, P. 2005. Yields Losses Associated With Peanut Mottle Virus (PtMtV) Infection on Peanut Plants Given Different Regimes of Urea And Potassium Chloride Fertilizers. Research Report, Agricultural Faculty, Tadulako University.

Šutič, D.D., R.E. Ford, and M.T. Tošič. 1999. Handbook of Plant Virus Diseases. CRC Press, Boca Raton.

Sward, R.J. and N.D. Hallam. 1976. Changes in fine structure of the potato meristem following heat treatment for virus eradication. Australian Journal of Botany 24(5): 597-605 (Abstract).

Ziems, A.D., L.J. Giesler, G.L. Graef, and L.C. Lane. 2001. Effect of bean pod mottle virus on soybean seed quality. APS Abstracts of Presentations, Phytopathology 91: S100.

Yang, Y., K.S. Kim, and E.J. Anderson. 1997. Seed transmission of cucumber mosaic virus in spinach. Phytopathology 87: 924-931.