Agroland: The Agriculture Science Journal, 2015 December 2(2) : 91–97 **ISSN : 2407 - 7585**
E-ISSN : 2407 - 7593

PROPAGATION OF LEMBAH PALU SHALLOT SOMATIC EMBRYOSAS EFFORTSTO PROVIDE GOOD QUALITY SEED

Maemunah[1)], Ramal Yusuf[1)], Hawalina[2)], Yusran[3)]

[1)] Lecturer and Researcher at Department of Agro-Technology Faculty of Agriculture.
University of Tadulako. Palu. E-mail : maemunah_tadulako@yahoo.co.id and ryusufus@untad.ac.id
[2)] Plant Biotechnology Laboratory Faculty of Agriculture. University of Tadulako. Palu. E-mail: hawalinak@yahoo.com
[3)] Science and Seed Technology Laboratory Faculty of Agriculture. University of Tadulako. Palu.
E-mail: Yusran_untad@yahoo.co.id

ABSTRACT

Shallot (*Allium wakegi Araki*c.v. Lembah Palu) is a horticulture commodity prospective as a very good business opportunity, since becomes the major ingredient of fried shallot, the most unique souvenir of Palu. The productivity however, is generally low due to lack in availability of good seed and seed production technology. The purpose of this study is to develop seed production technology through multiplication of somatic embryos. This assessment is the second part of research stages regarding the development of shallotsomatic embryo technology in Central Sulawesi. The first stage of the research is setrilization and callus induction of Lembah Palu shallot. This second stage is maturation dan germination of somatic embryos.The experiment was arranged as factorial completely randomized design, comprising of two factors. The first factor was 2,4-D concentration; consisting of three levels i.e 0.00, 0.25 and 0.50 mg/L. The second factor is kinetin concentration with the same levels as in 2,4-D. Each treatment combination was replicated three times, thus there were 27 experimental units. The result showed that addition of kinetin in MS basal media strongly affected the percentage of mature embryos and the percentage of mature embyos producing shoots, with the highest rate was obtained from the media fortified with 0,50 mg/L kinetin (26,913% and 43,318% respectively). Addition of 2,4-D to the MS basal media tended to inhibit the rate of regenerating shoot from the embryos. There is no interaction between 2,4-D and kinetin concentrations in affecting maturation and germination of the somatic embryos.

Key Words: Kinetin, propagation, shallot, somatic embryo, and 2,4-D.

INTRODUCTION

Shallot (*Allium wakegi Araki*c.v. Lembah Palu) is an important horticulture commodity for farmers in Palu, promising business opportunity, since becomes the major ingredient of fried shallot, the most unique souvenir of Palu. The productivity in Central Sulawesi however, has declined from year to year. Statistic datas showed in 2011, the productivity reached 7.84 tons per ha, butdropped gradually to4.12 tons per ha in 2012 and 3.37 tons per ha in 2013 (BPS, 2013).

One ofthe causes behind the decline is lack in availability of good seed and seed production technology (Maemunah, 2014). While seed as any type of planting material that is intended for use in producing a crop, i.e. either generative or vegetative, is one of the main factors influencing the successful of plant cultivation. The Food and Agriculture Organization of the United Nations (FAO), stated that the increase in impurities in a variety and decline of production about 2.6% per cropping cicle was mainly affected by the use of poor quality seeds (Erythrina, 2011).

The only alternative available for farmers is using poor quality seeds (Limbongan and Maskar, 2003), since farmers always rely much on the remainder of harvest yield inwhich the seed taken, often in small size. The quality of shallot seed may differ and depending on harvest

yield obtained by every farmer. Another constrain is this shallot does not produce flower, thus it can not produce true seed as the source of new planting materials (Maemunah, 2015). Conventionally, the farmers use the shallot bulbs as seed, while the bulb has certain dormant period, it needscertain duration of "hanging" for germination (Maemunah *et al*., 2015). Therefore, it is crucial to develop a vegetative fast propagation to provide good quality seeds of the shallot, such as propagation of somatic embryos through callus induction or embryogenesis technology. By this method, it can be produced huge numbers of planlets as new planting source in a shorter period, uniform, free of insect and disease, have the same potentials as their original plants and without considering the uncertainty of climate change (Basri, 2004 and Purnamaningsih, 2002).

Some researches on embryogenesis of *Allium sp*. has been done, such as Van der Valk *et al*. (1992), Handayani (1998), Tanikawa *et al*. (1998), Zheng *et al*. (1998), Mariani *et al*. (2003), Tanikawa *et al*. (2004), Tiwari *et al*. (2007), Nasim *et al.* (2010) and Ramakrishnan *et al*. (2013). Most of them used mature seeds as explant, very few of them used vegetative organ *i.e*. leaves, root tips and basal plate and none used bulb.

Using "Palu" shallot bulbs as explant was just started to evaluate the effect of 2,4-D and or coconut water on callus induction (Sorentina *et al*., 2013; Armila *et al*., 2014). The difficulties faced in using bulb as explant is high contamination due to direct contact between bulb and soil. Therefore, the previous experimental series of this research package was firstly focused on finding out a sterilization method, to encounter contaminated culture, followed by evaluation of 2,4-D effects on callus induction. The result showed that rinsing thebulbs in solution of 1g/L bactericide + 1g/L fungicide for 24 hours, followed by 10% and 5% Bayclin (commercial bleach with 5,25% NaOCl) for 10 and 5 minutes respectively, and then passing the bulbs through Bunsen flame before culture, gave the best result. The best concentration of 2,4-D to induce callus was 2 mg/L added to MS basal media, resulting in 91,67% callus formation with the fastest was 25,66 days after culture (Maemunah and Bustami, 2013).

In order to propagate shallot *in vitro* through organogenesis, the use of kinetin has been severily documented (Yunus, 2007). Kamstaityte and Stanys (2004) claimed that the highest micropropagation frequency using kinetin (1.9 to2.1 microshoots per explant) was obtained at a moderate (10.6 μM) concentration. The regeneration intensity (output of microshoots) was 68% higher using kinetin in comparison with BAP. In this research it was examined the effects of 2,4-D and kinetin on maturation of embryogenic calli obtained from previous callus induction using bulb as explant.

MATERIALS AND METHODS

Plant Materials. Explant in this experiment was the best callus obtained from the last experiment of callus induction (Maemunah dan Mirni, 2013). The calli looked fresh, friable, yellowish in colour, and globular in shape. Each explant was a clump of calli, about 3-4 mm in diameter consisting of about 15 embryos. Each experimental unit has three explants, thus, the total embryos for each experimental unit were 45.

Culture Media. The basal media used in this experiment was Murashige-Skoog (MS; Murashige and Skoog,1962), with 30% saccarose, fortified with 2,4-D (0.00, 0.25, or 0.50 mg per L) combined with kinetin (0.00, 0.25, or 0.50 mg per L), solidified with 2.0 g/L phytagel. The pH of each media was adjusted to 5,7 – 5,8 using NaOH 0.5 N or HCl 0.5 N, prior to auto claving at 121^{o}C, 17.5 psi for 15 minutes.

Experimental Design. This experiment was arranged as factorial completely randomized design, comprising of two factors. The first factor was 2,4-D concentration; consisting of three levels *i.e*0.00, 0.25 and 0.50 mg/L. The second factor is kinetin concentration

with the same levels as in 2,4-D. Each treatment combination was replicated three times, thus there were 27 experimental units. The percentage of mature embryos was counted six weeks after culture and the percentage of the mature embryos to germinate was counted two weeks later. The time when the embryos started to germinate was recorded until the six weeks of culture. Transfer of the explants to fresh media with the same composition was done every two weeks. The effect of the treatments was analyzed using Analysis of Variance and the mean differences between the treatments was analyzed with Duncan Multiple Range Test as in Gomez and Gomez (1995) and Hanafiah (2004).

Culture Condition. All the cultures in this experiment were incubated in a sterile room, placed under a fluorescence continous light source of 40 Watt (2500 lux) for 12 hours. Temperature of the room was retained at 25 ^{o}C with air conditioners. Any contaminated culture was soon removed from the incubation room, and then sterilized using autoclave, prior of cleaning by washing.

RESULTS

The Percentage of Mature Embryos. Analysis of variance on the percentage of mature embryos, *i.e* the embryos that regenerated to be bipolar embryos (mature embryos) showed that, kinetin concentrarion applied was highly significant in affecting the percentage of mature embryos 6 weeks after culture. While 2,4-D applied in that concentration and the interaction between the two plant regulators (PGRs) did not affect the percentage of mature embryos. The mean differences between the treatments of kinetin with the result of the DMRT at 1% level is shown in Table 1.

The Percentage of Mature Embryos to Germinate (Forming Shoots). Analysis of variance on the percentage of mature embryos that germinated, *i.e* the bipolar embryos that turned to be shoots showed that, kinetin concentration applied was highly significant in affecting the percentage of mature embryos 8 weeks after culture. While 2,4-D applied in that concentration and the interaction between the two plant regulators (PGRs) did not affect the percentage of mature embryos forming shoots. The mean differences between the treatments of kinetin with the result of the DMRT at 1% level is shown in Table 2.

The Time when The Embryos Started to Germinate. The datas were scored as in Table 3.

Analysis of variance on the time to germinate or forming shoots of the bipolar embryos, showed that, kinetin as well as 2,4-D concentrarions applied was highly significant in affecting the time to germinate or forming shoots, but the interaction between the two plant regulators (PGRs) did not affect the time to form shoots. The mean differences between the treatments of kinetin and of 2,4-D with the result of the DMRT at 1% level is shown in Table 4 and Table 5 seperately.

Table 1. The Percentage of Mature Embryos 6 Weeks After Culture

Treatment	Mean Percentage of Mature Embryos (%)*
Kinetin 0,00 ppm	2,960[b]
Kinetin 0,25 ppm	26,173[a]
Kinetin 0,50 ppm	26,913[a]

* Remarks : Means within Acolumn Followed by The Same Letter do not Differ Significantly using DMRT (P = 0.01).

Table 2. The Percentage of Mature Embryos *to Germinate (Forming Shoots)*

Treatment	Mean Percentage of Mature Embryos Forming Shoots (%)*
Kinetin 0,00 ppm	0.000[b]
Kinetin 0,25 ppm	43,220[a]
Kinetin 0,50 ppm	43,318 [a]

* Remarks : Means within Acolumn Followed by The Same Letter do not Differ Significantly using DMRT (P = 0.01).

Tabel 3. Data Score of Time to Germinate

Score	Time to Germinate
3	Germinateat the weeks fourth
2	Germinateat the weeks fifth
1	Germinateat the weeks six
0	Did not germinate

Table 4. Time When The Embryos Started to Form Shoot (Scored) as The Main Effect of Kinetin

Treatment	Mean Scored Time to Form Shoot
Kinetin 0,00 ppm	0,000[b]
Kinetin 0,25 ppm	2,000[a]
Kinetin 0,50 ppm	2,333[a]

* Remarks : Means within Acolumn Followed by The Same Letter do not Differ Significantly using DMRT (P = 0.01).

Tabel 5. Time When The Embryos Startedto Form Shoot (Scored) as The Main Effect of 2,4-D

Treatment	Mean Scorred Time to Form Shoot
2,4-D 0,00 ppm	1,778[a]
2,4-D 0,25 ppm	1,556[ab]
2,4-D 0,50 ppm	1,000[b]

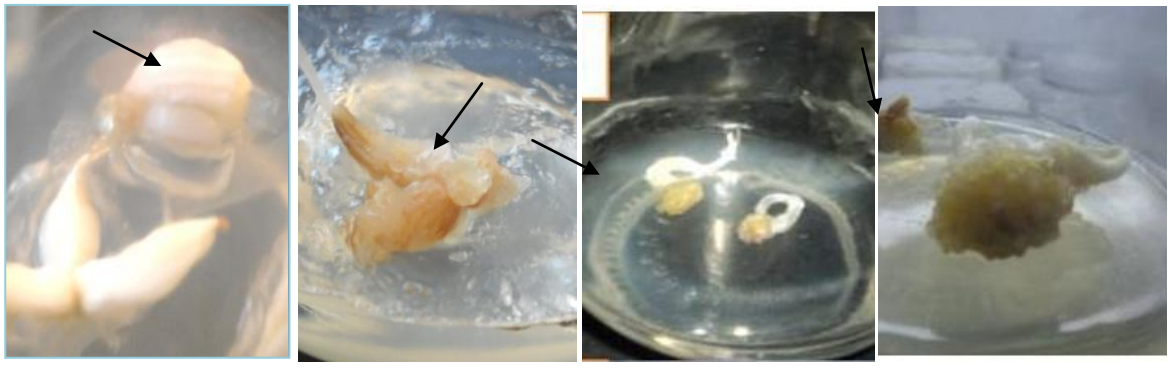
* Remarks : Means within Acolumn Followed by The Same Letter do not Differ Significantly using DMRT (P = 0.01).

DISCUSSION

In the process to propagate callus derived from Palu shallot bulb, development of the cells of this species (*Allium wakegi Araki* var. Lembah Palu) (Maemunah, 2015) in structure, was well documented and showed in Figure 1. The strutural changes showed the same pattern with those obtained by Tanikawa *et al*. (2004), in the development of onion (*Allium cepa* L.) cells. Initially, structures like calli were emerged over the bulb surface that were not in direct contact with media. These no apparent struture of calli, were white in colour, transparant, hyperhydrated, and watery. In the callus induction media (MS supplemented with 2 mg/L 2,4-D), the structures-like calli were firstly formed four days after culture (Figure 1a), some of them developed to be shoot-like strutures, white in colour or yellowish at the tips, but very weak, watery, abnormal and did not develop to be normal shoots (Figure 1b).

Globular calli, on the other hand, yellowish in colour, frible to slightly compact, were formed later, about 3-4 weeks after culture, initially around the basal plate of the bulb which has changed from its original shape (Figure 1c).

These type of calli are the embryogenic calli, which then develop to be shoots (Figure 1d).



a b c d

Figure 1.a. Bulb Surface Changing to Form Structure Like-Calli. b. Shoot-Like Structure, Abnormal from Watery Calli. c. Globular Callus Emerged Later on. d. Embryogenic Callias Explan Source.

Figure 2. A. Globular Embryos as Explant. B. Bipolar Embryos. C. Shoot of Watery Callus. D. Shoot of Friable Embryos.

The result of this experiment showed that addition of kinetin up to 0.50 ppm to the MS basal media, promoted the induction of globular embryos to regenerate into bipolar structures. The bipolar structures of the embryos then grew further to be micro shoots (Figure 2). The percentage of mature embryosand the embryos forming shoots, tended to increase as the kinetin consentration increase (Table 1 and 2). A similar result obtained in the experiment of onion suspension culture by Tiwari *et al*. (2007), where addition of kinetin 1 ppm was better in increasing the fresh weight and the relative growth of embryo compared to 0,50 ppm, meanwhile, concentration of 2.00 ppm tended to produce too compact callus which then died in the period of the research. Regeneration intensity (in the form of microshoots) was 68%, higher than those obtained when using BAP.A completely different respons reported by Patena *et al*. (1991) who studied shoot induction through organogenesis, stated that NAA-kinetin combination was not effective to induce shoot of garlic and also less effective for shallot, compared to NAA-BAP combination. To regenerate shoot from embryogenic calli of shallot, however, Ramakrishnan *et al*. (2013) stated that addition of BAP was essential, and the highest concentration tested (1,5 mg/L) was optimum with 28,7% shoot regeneration resulting in 2,9 shoots per callus. The previous results indicated that the potence to increase regeneration of shallot somatic embryo at the concentration higher than 0.50 ppm is reliable to try.

The use of kinetin in propagation of shallot through organogenesis has been severily reported. Kamstaityte and Stanys (2004) found that the highest frequency of micropropagation (1.9 - 2.1 microshoots per explant) using kinetin was obtained at middle concentration (10.6 μM). Based on molecular weight of kinetin which is 215,25 g/mol (Taji *et al*., 1997), the concentration is equal to 2,282 mg/L. In other experiment, Yunus (2007) reported that in 3 mg/L kinetin (without IAA) the shallot shoots were faster to emerge which was 2,67 days. In combination with kinetin, this experiment also evaluates 2,4-D effects on maturation and germinationof shallot embryos derived from bulb explant.

The use of 2,4-D in callus induction of shallot derived from true seed as explant, such as done by van der Valk *et al*. (1992) using zygotic embryos, Saker(1997) and Zheng *et al*. (1998) using mature embryos, Ramakrishnan *et al*. (2013) using shoot tips is common as they showed that 2,4-D was suitable to induce embryogenic callus. Using "Palu" shallot with bulbs as explant, Sorentina *et al*. (2013) and Armila *et al*. (2014) were succeed to induce embryogenic callus, as also has been done by Maemunah and Bustami (2013). But in the case of regenerating embryos to be shoots in this experiment, the use of 2,4-D seems to be less significant. In the absence of kinetin, lower concentration of 2,4-D promotes faster embryo to germinate, indicating inhibition of the PGR on the germination of the shallot embryo (see Table 5). The similar

responses were obtained on micropropagation of shoot through embryogenesis, where addition of IAA (an auxin) significantly inhibited shoot induction (Yunus, 2007). It was presumed that the inhibition occured as the side effect of auxin which promotes formation and growth of roots instead of shoots.

Changing media composition from the existance of high 2,4-D concentration (2-4 mg/L) for callus induction (Sorentina *et al*., 2013; Armila *et al*., 2014 and Maemunah and Bustami, 2013), to the composition without auxin in shoot induction media (Yunus, 2007 and this experiment), indicates that in every stage or phase of cell growth *in vitro*, the composition of PGRs in the media strongly affected the pattern of the growth. Ramakrishnan *et al*. (2013) claimed that relatively small differences in medium composition caused completely different regeneration responses of explants. Generally, Wattimena *et al*. (1991) stated that proliferation of axillary shoots needs cytokinin (such as kinetin, BAP, etc.) in a high concentration without auxin, or with auxin in a very low concentration.

Compared to the result reported by Ramakrishnan *et al*. (2013) on shoot regeneration (28,7%), the results of using kinetin in this experiment were comparatively high enough (26,913% mature embryos and 43,318% mature embryos producing shoots). However, with the results obtained from shoot regeneration through suspension culture, reported by Tiwari *et al*. (2007) (68% regeneration intencity in the form of micro shoots), the improvement of shoot regeneration technology on Palu shallot embryos derived from bulb in solid media, still needs to study further.

CONCLUSION

From the finding and discussion, it can be concluded that addition of kinetin up to 0.50 ppm to the MS basal media, promoted maturation and germination of the somatic embryos of Palu shallot, resulting in 26,913% mature embryos and 43,318% embryos producing shoots.The present of 2,4-D in the media inhibited the maturation and germination of the somatic embryos.

REFERENCES

Armila, P NK., Mirni Ulfa Bustami, and Zainuddin Basri, 2014. *Sterilization and Callus Induction of Local Palu Shallot (Allium ascalonicum* L.*) In Vitro*. J. Agrotekbis. Vol. 2 No. 2.

Basri, Z., 2004. *Plant Tissue Culture*. Tadulako University Press. Palu.

BPS, 2013. Indonesia Statistic Center Institute.

Erythrina.,2011. Seeding and Onion Cultivation. National Seminar on Agriculture Technology Innovation to Support Sustainable Food Securityand Rice Self Sufficience at North Sulawesi. Pg: 74- 84.

Gomez, K.A. and A.A.Gomez. 1995. *Statistic Procedure for Agriculture Experiment*. Translation: Endang Syamsuddin and Justika S. Baharsjah.UI-Press. Jakarta. Pg: 342-360.

Hanafiah, K.A., 2004. Experimental Design. 3rd Ed. PT. Raja Grafindo Persada. Jakarta.

Handayani, T.T., 1998. *Callus Induction of Onion* (*Allium cepa* L.) *In Vitro*. Experimental Final Report. Faculty of Mathemathic and Science. Lampung University.

Kamstaityte, D. and Stanys, V., 2004. *Micropropagation of Onion* (*Allium cepa* L.). Acta Universitatis Latviensis, Biology. Vol. 676, pp. 173–176.

Limbongan, J., and Maskar, 2003. *Potential Development and Availability of The Technology on Lembah Palu Shallot in Central Sulawesi*. Journal of Agricultural Research and Development. Vol. 22(3).

Maemunah, 2014. *Seed Production and Local Approach in Improving Seed Quality of Local Palu Shallot*. Proceeding on National Seminar"PERHORTI, Malang 5-7 November 2014 ISBN 978-979-508-017-6 226.

Maemunah, 2015.*Improving Technology on Lembah Palu Shallot Seed Production*. Ph.D Thesis. Ph.D Program at Agriculture Science. Brawijaya University. Malang.

Maemunah and Bustami, M.U., 2013. *Propagation Strategies of Lembah Palu Shallot Through Tissue Culture to Provide Good Quality Seed*. Final Report of Competitive Reward Research 2013. Tadulako University.

Maemunah, T.Wardiyati, B.Guritno and A. N. Sugiarto, 2015. *The Influence of Storage Area, Storage Method and Seed Quality Character on the Quality of Shallot Seed*. Int. J. Adv. Res. Biol. Sci. 2(1): (2015): 158–164.

Mariani T.S., Hiroshi Miyake, Rizkita Rachmi Esyanti and Ida Nurwendah, 2003. *Effect of 2,4-D on Indirect Somatic Embryogenesis and Surface Structural Changes in Garlic* (*Allium sativum*L.) *cv. Lumbu Hijau*. Journal of Mathemathicand Science. Vol. 8 No. 4. December 2003.pg 133 – 139.

Nasim S.A., Mujib, A., Rashmi Kapoor, Samar Fatima, Junaid Aslam and Mahmooduzzafar, 2010. *Somatic Embryogenesis in Allium sativum* L. *cv. Yamuna: Improving Embryo Maturation and Germination with PGRs and Carbohydrates*. *Anales de Biología* 32: 1-9.

Patena, L.F., Bernardita A dela Rosa and Teresita L. Rosario, 1991. *In Vitro Respon of Garlic* (*Allium sativum* L.) *and Shallot* (*Allium ascalonicum* L.) *to 6-Benzylaminopurine, Kinetin, 2-Isopentenyladenine, 1-Naphthaleneacetic Acid and Mannitol*. Phillip J. Crop Sci. 26(1) Pg 25-28.

Purnamaningsih R., 2002. Plant *Regeneration Through Somatic Embryogenesis Related Genes*. Agrobio. 5(2): 51-58.

Ramakrishnan, M., S. Antony Ceasar, V. Duraipandiyan, Melvin A. Daniel and S. Ignacimuthu, 2013. *Efficacious Somatic Embryogenesis and Fertile Plant Recovery from Shoot Apex Explants of Onion* (*Allium cepa* L.). *In Vitro Cell. Dev. Biol*.

Sorentina, Melisa S., Haliani, Muslimin Mand I Nengah Suwastika, 2013. *Callus Inducton of Local Palu Shallot* (*Allium ascalonicum* L.) *in MS Media Supplemented with 2,4-D and Coconut Water*. Online Journal of Natural Science FMIPAVol. 2 No 2.

Taji, A.M., William A. Dodd and R.R. Williams, 1997. *Plant Tissue Culture Practice*.3th*Edition*. The University of New England. Armidale. NSW Australia.

Tanikawa,T., Masatoshi Tagaki and Masahiko Ichii, 1998. *Varietal Differences in Plant Regeneration from Solid and Supension Cultures in Onion* (*Allium cepa* L.). J. Japan Soc. Hort. Sci. 67 (6) : 856 – 861.

Tanikawa,T., Hisaaki Uragou, Masatoshi Tagaki, and Masahiko Ichii, 2004. *Histological Investigation of Plant Regeneration Process in Onion (Allium cepa) Calli*. J. Japan. Soc. Hort. Sci. 73 (4) : 340 – 342.

Tiwari *et al*. (2007), Tiwari, S, M.K., Tripathi, U.K., Khare and Rana, 2007. *Initiation of Embryogenic Suspension Culture and Plant Regeneration in Onion*. Indian J.Biotechnol., 6: 100-106.

Van der Valk., O.E. Scholten, F. Verstappen, R.C. Jansen and J.J.M. Dons, 1992.*High Frequency Somatic Embryogenesis and Plant Regeneration of Zygotic Embryo-Derived Callus Cultures of Three Allium Species*. Plant Cell, Tissue and Organ Culture. 30: 181 – 192.

Zheng Sijun, Betty Henken, Eri Sofiari, Evert Jacobsen, Frans A. Krens and Chris Kik, 1998.*Factors Influencing Induction, Propagation and Regeneration of Mature Zygotic Embryo-Derived Callus from Allium cepa*. Plant Cell, Tissue and Organ Culture. 53**:** 99–105.

Yunus,A., 2007. *The Effect of IAA and Kinetin on Explant Growth of Shallot*(*Allium ascalonicum* L.) *In Vitro*. Journal of Acta Agrosia.Special Edition.No. 1 Pg. 53-58.