Initial Studies on *In Vitro* Germination and Early Seedling Growth of *Nepenthes truncata* Macf.

Eufemio T. Rasco Jr. and Mary Ann D. Maquilan • College of Science and Mathematics • University of the Philippines in Mindanao • Bago Oshiro • Davao City 8000 • Philippines • etrasco@mozcom.com

Keywords: cultivation, *Nepenthes truncata*, tissue culture.

In order to develop and establish a protocol for *in vitro* seed germination and early seedling growth for *Nepenthes truncata* Macf., we designed a study to test the effects of different sterilization methods as well as seed soaking treatment, culture medium, and salt concentration.

A series of sterilization trials was performed to determine the minimum amount of sterilization that could produce clean cultures without reducing seed viability. Successive treatments of 3% hydrogen peroxide, 10% Zonrox® bleach and 70% Johnson's® isopropyl alcohol provided the most satisfactory results on sterilization without reducing the seed viability. This protocol resulted in 90.8% sterilization and 83.7% germination based on a sample size of 520 cultures containing 10 seeds each; the seeds were collected from the wild and stored for two weeks in silica gel prior to sowing. Seed pretreatments include: soaking in 10% table sugar solution and hardening involving 24 hours of soaking in distilled water and 24 hours of air-drying. All the culture media tried con-

Figure 1: Sample 125 ml Erlenmeyer flasks topped with aluminum foil and paper sheets, and 200 ml food jars with metal twist-off closures from the *in vitro* germination experiments. Seedlings in the Erlenmeyer flask show advanced growth, possibly due to the greater light transmission allowed by the vessel and more efficient exchange of gases between the external and internal environments allowed by the type of closure.

tained the respective basal salts plus full Murashige and Skoog (MS) iron source and vitamin supplements. Two types of culture vessels were used in the experiments; 125 ml Pyrex® Erlenmeyer flasks capped with paper sheets and, interior to that, unvented aluminum foil secured by rubber bands, and 200 ml food jars with unvented metal twist-off closures. Each vessel contained 25 ml of the specified medium. The cultures were maintained under 25±2°C temperature and 14 hours light which were provided by 40 W fluorescent tubes at 20 cm above culture level.

The main and interaction effects of seed soaking treatments, medium, and salt concentration on the *in vitro* germination of *N. truncata* seeds were studied based on the initial germination percentage (IGP), germination rate (GR) and final germination percentage (FGP). The IGP was recorded after 18 days in the germination medium while GR and FGP yields were taken after 74 days. To document the progress of germination percentage yields in each experimental unit, measurements were recorded every 7 days and the GR values were calculated based on the formula:

$$GR=(FGP-IGP)/T,$$

where T is the number of days that elapsed between the date when the maximum FGP was achieved and the date that the IGP was recorded.

To determine the most suitable medium for *N. truncata* seedlings, the effects of different media on the growth and development were evaluated considering morphological properties such as pitcher formation, leaf condition, shoot length, and root length that were assessed using devised scoring systems for each parameter.

Our results indicate that soaking in 10% table sugar solution significantly facilitated early germination for both flask and jar cultures. The highest IGP was attained in flasks treated by the com-

Table 1: The effects of seed soaking treatment, medium, and concentration on final germination percentage of *Nepenthes truncata* seeds sown in flasks and jars.[1]

		Final Germination Percentage (FGP)			
		Erlenmeyer flask		Food jar	
Medium	Concentration	Sugar solution	Hardening	Sugar solution	Hardening
Control	(no nutrients)	81	86	77	81
MS	1/4	83^{cd}	94^{ab}	76^{ab}	87^{a}
	1/2	90^{abcd}	94^{ab}	79^{ab}	78^{ab}
	3/4	84^{bcd}	88^{abcd}	80^{ab}	83^{ab}
	1	84^{bcd}	94^{a}	79^{ab}	86^{a}
	Mean	85	92	78	84
WPM	1/4	94^{abc}	91^{abcd}	72^{ab}	74^{ab}
	1/2	85^{bcd}	88^{abcd}	72^{ab}	72^{ab}
	3/4	86^{abcd}	85^{abcd}	77^{ab}	65^{b}
	1	84^{bcd}	87^{abcd}	79^{ab}	73^{ab}
	Mean	87	88	75	71
KC	1/4	87^{abcd}	94^{abc}	82^{a}	86^{a}
	1/2	91^{abcd}	91^{abcd}	88^{a}	80^{ab}
	3/4	86^{abcd}	94^{ab}	82^{ab}	82^{ab}
	1	95^{a}	90^{abcd}	82^{ab}	72^{ab}
	Mean	90	92	84	80
	Grand Mean	87	90	79	78
	CV[2]	15		20	

[1]Values within flask or jar treatments followed by common letter(s) are not significantly different using Duncan's Multiple Range Test at the 5% level.

[2]Coefficient of variation, in percent.

Table 2: The effects of sowing medium on the germination rate of *Nepenthes truncata* seeds sown in flasks and jars.[1]

Medium	Concentration	Germination Rate (GR) (% germination/day) Erlenmeyer flask	Food jar
Control	(no nutrients)	1.3	1.2
MS	1/4	1.3^{e}	1.2^{abc}
	1/2	1.6^{cde}	1.2^{abc}
	3/4	1.5^{cde}	1.2^{ab}
	1	1.8^{b}	1.3^{a}
	Mean	1.6	1.2
WPM	1/4	1.4^{de}	1.1^{abc}
	1/2	1.5^{cde}	1.1^{bc}
	3/4	1.6^{bcde}	1.0^{c}
	1	1.5^{cde}	1.2^{abc}
	Mean	1.5	1.1
KC	1/4	1.7^{bc}	1.2^{abc}
	1/2	1.4^{de}	1.2^{abc}
	3/4	1.6^{abc}	1.2^{abc}
	1	1.6^{bcd}	1.1^{bc}
	Mean	1.6	1.2
	Grand Mean	1.5	1.2
	CV[2]	16	16

[1]Values within flask or jar treatments followed by common letter(s) are not significantly different using Duncan's Multiple Range Test at the 5% level.
[2]Coefficient of variation, in percent.

bination of seed soaking in sugar solution and Woody Plant Medium (WPM) with one-fourth salt concentration (17.5%) followed by Knudson C (KC) medium with three-fourth and full salt concentrations (14% and 13.8% respectively). MS medium with one-half and three-fourth salt concentrations gave comparably higher IGP (13% and 12.5%). In terms of FGP (Table 1), relatively strong interaction effects of soaking treatment and medium were observed in both flask and jar cultures while the concentration effects were weak. The best treatment for achieving highest FGP was a combination of hardening treatment (one cycle of soaking and drying) and MS (92.4%) or KC (92.2%) using flasks. On the other hand, GR (Table 2) was significantly affected by medium (for jar cultures) and medium and concentration interactions (for flask cultures). Sowing seeds in MS with full salt concentration using flasks resulted in the highest GR (1.83%). The results provided evidence that subjecting seeds to high osmotic concentrations does not necessarily decrease GR nor decrease the FGP for *N. truncata* seeds. This is contrary to earlier conclusions that GR is inhibited under high salinity conditions (Ayers, 1952, as cited in Arteca, 1996), and that GR decreases with decreasing external water potential (Hadas, 1976, and Hadas & Russo, 1974, as cited in Levitt, 1980). Toxicity because of the highly osmotic medium, however, was manifested in seedlings.

Germination time generally was within the range of 18 to 74 days (by which time the FGP had in general stabilized), and we note this period is shorter than the range of 28 to 180 days reported by Bickell (2000). FGP was higher and seedling growth was more advanced in flasks than in jars. The responses were probably affected by the differences in light transmission brought about by the type of glass as well as exchange of gases between the external and internal medium brought about by the type of closure. Erlenmeyer flasks probably permitted greater light transmission and the effects are manifested by the direction of shoot growth, leaf condition, as well as pitcher formation (see Figure 1).

Table 3: The effects of medium and concentration on pitcher formation, leaf condition, shoot length, and root length of *Nepenthes truncata* seedlings in flasks.[1]

Medium	Concentration 1/4	1/2	3/4	1	Mean
			Pitcher formation[3]		
Control					2.4
MS	1.8^{ef}	3.1^{c}	1.1^{fg}	1.0^{g}	1.7
WPM	3.0^{cd}	2.4^{de}	3.2^{bc}	1.6^{fg}	2.5
KC	4.0^{a}	3.8^{ab}	3.0^{cd}	2.4^{d}	3.3
Mean	3.0	3.1	2.4	1.6	2.5
CV[2]	27				
			Leaf condition		
Control					2.4
MS	3.8^{b}	4.6^{a}	2.4^{c}	1.1^{d}	3.0
WPM	2.1^{c}	2.1^{c}	2.4^{c}	2.2^{c}	2.2
KC	5.0^{a}	5.0^{a}	4.9^{a}	5.0^{a}	5.0
Mean	2.9	3.1	2.4	1.6	2.5
CV[2]	16				
			Shoot length		
Control					3.5
MS	3.1^{e}	4.5^{ab}	1.1^{g}	1.0^{g}	2.4
WPM	4.2^{bc}	2.4^{f}	3.5^{de}	2.0^{f}	3.0
KC	5.0^{a}	5.0^{a}	5.0^{ab}	4.0^{cd}	4.6
Mean	4.1	4.0	3.1	2.2	3.4
CV[2]	19				
			Root length		
Control					2.2
MS	1.0^{e}	1.6^{bc}	1.0^{e}	1.0^{e}	1.2
WPM	2.8^{a}	2.6^{a}	2.6^{a}	1.4^{d}	2.4
KC	1.8^{b}	1.2^{de}	1.0^{e}	1.0^{e}	1.3
Mean	1.9	1.8	1.6	1.2	1.6
CV[2]	24				

[1]Values within a parameter followed by common letter(s) are not significantly different using Duncan's Multiple Range Test at 5% level.

[2]Coefficient of variation, in percent.

[3]Data are provided for this and other rankings in dimensionless units, as described in the text.

Seedling growth and development were further evaluated based on pitcher formation, shoot length, leaf condition and root length. A scoring system of 1-5 was developed to rate each character. Pitcher formation was rated as follows: (1)absence of pitchers in all seedlings, (2)presence of slightly developed pitchers in majority of the seedlings, (3)equal distribution of seedlings with well-formed and slightly-formed pitchers, (4)presence of well-formed pitchers in majority of the seedlings, (5)presence of well-formed pitchers in all seedlings. Shoot length was rated as follows: (1)short stems without pitchers, (2)long stems without pitchers, (3)stems are longer than the pitchers, (4)stems are of equal length with the pitchers, (5)stems are shorter than the pitchers. Leaf condition was rated as follows: (1)incidence of chlorosis (white or brownish leaves), (2)incidence of deterioration such as yellowing and appearance of necrotic spots, (3)pale green and narrow leaves, (4)pale green but broad leaves, (5)dark green and broad leaves. Finally, root length was rated as fol-

lows: (1)root formation is negligible, (2)root structures are visible as tiny spots, (3)intermediate between (2) and (4), (4)roots are longer but shorter than (5) and appear thick due to root hairs (rare occurrence), (5) roots are extremely long and thin without root hairs (rare occurrence). The values for the scores are given in Table 3.

Root formation was inhibited in MS and KC. Avoidance to osmotic stress, water and nutrient deficit, and consequent disturbance in photosynthesis and respiration offers possible explanations to the inhibition of root growth in seedlings in MS. In KC, however, root inhibition was brought about by more active shoot differentiation that diverted the expenditure of energy to shoot growth rather than root formation due to absence of ion stress.

In this study, KC at one-fourth to three-fourth salt concentrations enhanced growth and development in all cultures. The survival and growth of *N. truncata* seedlings were drastically decreased by the high salt concentration in MS. Seedlings exposed to three-fourth up to full strength MS salts and one-fourth to one-half concentration of WPM salts showed severe abnormalities (chlorosis, root rotting, and browning of stems). The rest of the treatments (except those in KC) also showed abnormalities but they evolved more slowly. These effects were probably caused by ion stress (Levitt, 1980) although the scope of the study could not substantiate such claim. To be able to interpret these observations and to determine specifically the cause of such effects, extensive studies on mineral nutrition for *in vitro* cultures of *N. truncata* should be conducted.

In order to achieve faster and more synchronized germination for *N. truncata* seeds, applying the hardening treatment is recommended. This treatment can promote faster seedling growth and better transplant survival. However, this treatment can be combined with soaking in sugar solution to achieve early germination as well. The most satisfactory results with *in vitro* seed germination can be achieved with KC using the sterilization procedures developed in this study. Adjustments to the medium can be made to suit the germination conditions. Among all the media tested, KC produced the healthiest seedlings, which can be good source plants for mass propagation or for acclimatization.

Acknowledgment: The authors are grateful to: the Bureau of Plant Industry, Bago Oshiro, Davao City 8000, Philippines through Mr. Orlando Pascua (OIC Center Chief), and the Tissue Culture Lab personnel, especially Mr. Celso Carreon (Lab Supervisor) for the shared facilities and deployment of skills; and Ms. Bilma Fuertes for providing the needed plant and seed materials.

References:

Arteca, R. 1996. Plant Growth Substances: Principles and Applications. Chapman & Hall, USA. pp. 104-120.

Ayers, A. 1952. Seed germination as affected by soil moisture and salinity. Agron. J. 44: 82-84. (cited in Arteca, 1996)

Bickell, A. 2000. *Nepenthes*. http://www.webhome.idirect.com/~bickell.html. Accessed 2000, April 28.

Hadas, A. 1976. Water uptake and germination of leguminous seeds under changing external water potential in osmotic solutions. J. Exp. Bot. 27: 480-489. (cited in Levitt, 1980)

Hadas, A., and D. Russo. 1974. Water uptake by seeds as affected by water stress, capillary conductivity, and seed-soil water contact: II. Analysis of experimental data. Agron. J. 66: 647-652. (cited in Levitt, 1980)

Levitt, J. 1980. Responses of Plants to Environmental Stress. Vol. II. Water, Radiation, Salt, and Other Stresses. Academic Press, Inc., N.Y., USA. pp. 27-28, 148-152, 378-380.

LOOKING BACK: CPN 25 YEARS AGO

In June 1979, James T. Robinson discussed the value and use of cultivar descriptions, and challenged horticulturists to use cultivar names to avoid the proliferation of poorly documented, confusing plant names: "What is needed now is the complete cooperation of both commercial and private CP growers for the proper naming and registration of CP cultivars. We have a fine opportunity to apply nomenclatural stability to the plants in our field of interest. Let's not let it escape us." Unfortunately, it is this editor's (BR) opinion that we have let the opportunity escape us almost entirely. Even though we now have an official carnivorous plant cultivar registration authority (the ICPS!), growers are still sloppily naming plants without registering them—the confusion continues!