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In vitro propagation of *Gladiolus dalenii* from the callus through the culture of corm slices

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ABSTRACT

Production of sufficient numbers of plants of a unique genotype is possible using *in vitro* culture system. In this study, the effects of various concentrations and combinations of plant growth regulators for *in vitro* regeneration of gladiolus were used with corm fractions as explants. Thus, an efficient *in vitro* propagation method was developed for *Gladiolus dalenii* using corm slices as explants. The highest rate of 90% explants for callus induction was observed on Murashige and Skoog (MS) medium supplemented with 7.5 mg/L napthaleneacetic acid after culture initiation of 90 days. Calli responded to form well-developed shoots on transferring to medium containing MS ± 0.5 mg/L benzylaminopurine ± 0.5 mg/L kinetin, in which 70% of the cultured calli responded to produce shoots. The average number of shoot per culture calli was 20 ± 2.40, and the average shoot lengths of 4.50 ± 0.45 cm were achieved in this medium after culture of 60 days. Recurrent callus induction and continuous plant regeneration were also observed in the same medium during the passage of shoot induction period. Shoots rooted well when they were excised individually and implanted on half-strength MS medium supplemented with 2.5 mg/L indole-3-butyric acid, which resulted 90% shoot induced roots after culture of 30 days. The average number of root per shoot was 10 ± 1.20, and the average root length of 5.5 ± 0.70 cm was observed in this medium. 80% of the *in vitro* raised plantlets were survived in the natural environment.

KEY WORDS: Callus induction, *Gladiolus dalenii, in vitro* corm culture, regeneration

INTRODUCTION

In vitro mass propagation technique would ensure true to type and rapid multiplication of disease free propagules and such can be used as super elite variety(s) for the quick spread. An efficient *in vitro* regeneration technique also provides economic advantages for the propagation of a particular crop such as gladiolus, a beautiful flowering plant which relates expression of love and symbol of peace. Propagation by the conventional method is a slow process, creates a seasonal barrier, and pathogen keeps on accumulation generation after generation which reduces yield and quality of flower and also generates insufficient propagules. An efficient propagation system could overcome variability and meet the increasing demand of propagules for the production of gladiolus which is an exporting plant in Bangladesh. Moreover, the establishment of a plant regeneration system through direct organogenesis or via callus is also a prerequisite to further *in vitro* genetic manipulation of the cultivar through somaclonal variation, *in vitro* mutagenesis and genetic transformation. The demand for disease-free planting materials is increasing day-by-day and crop like vegetatively propagated plant is an appropriate means to use *in vitro* culture technique to produce propagules.

People in the globe are realized that flowers enhance the quality of life and human feelings with more than thousand words or other gifts. This leads to increase the use of flowers and ornamental plants. *Gladiolus dalenii* is a popular and potential ornamental herbaceous cut flower that belongs to the family Iridaceae. The genus gladiolus includes 180 species with more than 10,000 cultivars of which about 20 are grown for commercial purposes (Sinha and Roy, 2002). The plant is developed annually from axillary buds on the corm which is a compressed

thickened stem. A new corm is formed annually by swelling of the basal internodes of the inflorescence stalk before and during flowering. A large corm is capable of producing 25-200 cormels depending on cultivars and culture conditions (Sinha and Roy, 2002).

A number of species are rising every year through hybridization with a view to extending vase life, producing novel colors, improve florets arrangement on the spike, and prolong the flowering period. The species of gladiolus have great demand with horticultural value and are cultivated all over the world. It is mainly propagated vegetatively by corms and cormels (Ziv and Lilien, 1990; Bose *et al*., 2003). This traditional method of propagation is limited by the low multiplication rate of corms, physiological dormancy of the corm and cormels and also other problems of corm rot during storage (Priyakumari and Sheela, 2005), non-availability of a large quantity of propagules for commercial cultivation (Singh and Dohare, 1994), fusarium corm rot disease, viral, and some other diseases (Sinha and Roy, 2002; Roy and Kabir, 2006). To overcome those variabilities, plant tissue culture method is an attractive alternative approach.

Micropropagation techniques increase multiplication rates (Novak and Petrun, 1981; Takagama and Misawa, 1982; Takagama and Misawa, 1983; Van *et al*., 1990). It also had been provided propagules free from viruses and other pathogens (Blom and Aartrijk, 1985; Van *et al*., 1990). In addition, plant cells or tissue cultured *in vitro* is capable of regenerating whole plants of which biotechnology affording a novel method for mass propagation (Murashige, 1977) with clonal uniformity (Iqbal *et al*., 2003). Moreover, this technique provides propagules from an elite variety(s) round the year. There were number of reports available pertaining to *in vitro* propagation of gladiolus (Rao *et al*., 1991; Dantu and Bhojwani, 1992; Sen and Sen 1995; Begum and Hadiuzzaman, 1995; Churrikova and Barykina, 1995; Grewal *et al*., 1995; Ziv *et al*., 1997; Gupta and Sehgal, 1997; Misra and Singh, 1999; Hussain *et al*., 2001; and Pathania *et al*., 2001; Prasad and Gupta, 2006). Various results have also been reported for the role of cytokinins in plant regeneration from callus initiated from different organs of gladiolus such as inflorescence stalk (Ziv *et al*., 1970), apical meristem (Logan and Zettler, 1985), axillary buds (Lilien and Kochba, 1987) basal leaves, and cormel slices (Kamo, 1994).

G. dalenii is a good consumer preference cut flower due to its attractive and colorful florets. Therefore, the development of efficient tissue culture protocol is necessary for mass propagation, conservation, and genetic improvement of this important ornamental plant. Thus, the present study was undertaken to determine the appropriate concentrations of growth regulators for callus induction and regeneration into plantlets of locally cultivated *G. dalenii* using corm slices as explants.

MATERIALS AND METHODS

Corm of *G. dalenii* was collected from the local farmer and planted at the Plant Biotechnology and Genetic Engineering Division experimental field at Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh.

A 1-month-old young corm was collected from the experimental field and used for the present study. The outer layer was removed from the corm and subsequently washed thoroughly with the detergent "Trix" and kept for 20 min under running tap water to eliminate dirt and organisms. The corms were then surface sterilized by treating with an aqueous solution of 0.1% mercuric chloride accompanied with 2 drops of "tween 20" for 10 min in the laminar air flow cabinet under aseptic conditions. Rinsing was done 4 times with sterile distilled water. Sterilized corms were then section longitudinally and cultured on MS with different concentrations of 2,4-D and napthaleneacetic acid (NAA) alone and also different concentrations and combinations of benzylaminopurine (BA) ± NAA and BA ± kinetin (Kin) for callus induction. Proliferated callus derived from the corm segments were transferred to the media containing MS supplemented with different concentrations of BA singly and in combinations with BA ± NAA and BA ± Kin for the regeneration of callus. Regenerated strong and healthy shoots were excised individually and transferred to the half-strength MS basal media supplemented with different concentrations of indole-3-butyric acid (IBA), IAA, and NAA for root induction. The sucrose (table sugar) concentration was used 30 g/L and the pH of the media adjusted to 5.8 prior to autoclaving. Cultures were incubated at 26 ± 2°C with a 16 h illumination of 21.8 µmol/cm^2/s provided by cool-white fluorescent tubes. Data were collected on different characters at day 90 for callus induction from corm segments and at day 60 for shooting from the callus and rooting of shoots at day 30. Observations on culture were carried out daily. The experiments were arranged in a completely randomized design with three replications for each treatment and five explants per replication. Each experiment was repeated twice. A descriptive analysis was carried out using the recorded data. Each value represents the mean ± standard errors. *In vitro* raised plantlets were removed from culture vessels, washed thoroughly to remove traces of nutrient

medium, transferred to polybags, and placed outdoor condition for acclimatization.

RESULTS AND DISCUSSION

The experiment was carried out to establish a suitable protocol for large scale *in vitro* regeneration and genetic improvement of locally cultivated *G. dalenii* using corm segment as explants with the interaction of different concentrations and combinations of plant growth regulators (Table 1). The callus induction potential from corm segment explants were found differently due to the different concentrations, combinations, and growth regulators used. The most effective results of 90% explants showed potential callus on medium containing MS ± 7.5 mg/L NAA within the culture period of 90 days (Figure 1a and b).

Calli responded to form well-developed shoots on medium containing MS ± 0.5 mg/L BA ± 0.5 mg/L Kin within the culture period of 60-day (Figure 1c-e). The average number of shoot per culture calli was 20 ± 2.40, and the average shoot length of 4.50 ± 0.45 cm was observed in this medium culture after 60 days (Table 2). Recurrent callus induction and continuous plant regeneration were also observed in the same medium during the passage of shoot induction period. The highest rate of callus formation from corm section explants of *Gladiolus* using NAA and callus regeneration was obtained when implanted on BA ± NAA was reported (Emek and Erdag, 2007). This is partially in agreement with the present investigation. Compact calli were observed in NAA supplemented media using various cultivars of gladiolus (Kamo *et al*., 1990). Similar results were reported by Kumar *et al*. (1999) when working with *Gladiolus hybridus* Hort. variety.

In this regard, the result from our study confirms the findings of them. Aftab *et al*. (2008) achieved callus from leaf explants using different concentrations of NAA and 2,4-D alone. They have found rhizogenesis and shoot organogenesis occurred rarely. This clearly indicates that morphogenesis depends on the nature of callus and the explant used. Good responses toward callus induction using NAA ± BA and 2,4-D alone and regeneration into plantlets using BA or Kin alone was reported (Sinha and Roy,2002) from slices of cormel sprouts of *Gladiolus primulinus* cv. golden wave. In our study, we found that combination of BA ± Kin showed excellent performance for callus regeneration which might be due to the effect of genotype. Kamo (1994) observed only actively growing regenerable callus of gladiolus using NAA. Our results also reveal the fact in forming callus and regeneration into plantlets using NAA. Rooting response differed according to the concentration of different auxins used (Table 3). Within the auxins, IBA was found the most optimum

Table 1: Effects of different concentrations of 2,4-D, NAA, NAA±BA, and BA±Kin with MS media on callus induction of orange color gladiolus (*Gladiolus dalenii*) using corm segment as explants at 90 days

Hormone supplements (mg/L)	Percentage of explants induced calli	Nature of response
2,4-D		
0.5	20	Yellowish and noduler callus
1.0	70	Yellowish, noduler, and embryo-like structure
1.5	30	Yellowish and noduler callus
2.0	-	-
2.5	-	-
NAA		
2.5	-	-
5.0	20	Yellowish and noduler callus
7.5	90	Yellowish, globuler, and embryogenic callus
10.0	30	Yellowish and noduler callus
12.5	-	-
NAA±BA		
1.0±0.2	-	-
2.0±0.2	50	Greenish and noduler callus
3.0±0.2	30	Greenish and noduler callus
4.0±0.2	-	-
5.0±0.2	-	-
BA±Kin		
0.1±0.5	50	White and watery callus
0.2±0.5	50	White and watery callus
0.3±0.5	50	White and watery callus
0.4±0.5	50	Creamy and globuler callus
0.5±0.5	70	whitish, globuler, and embryogenic

NAA: Napthaleneacetic acid, BA: Benzylaminopurine, Kin: Kinetin, MS: Murashige and Skoog

Table 2: Effects of different concentrations of BA, BA±NAA, and BA±Kin with MS media on shoot induction from embryogenic callus at 60 days

Hormone supplements	Percentage of calli responded to form shoots	Average number of shoot/cultured calli Mean±SE	Average shoot length (cm) Mean±SE
BA			
0.5	20	1.0±0.20	2.5±0.30
1.0	-	-	-
1.5	-	-	-
2.0	-	-	-
2.5	-	-	-
BA±NAA			
0.1±0.2	-	-	-
0.2±0.2	-	-	-
0.3±0.2	40	2.0±0.40	2.75±0.10
0.4±0.2	-	-	-
0.5±0.2	-	-	-
BA±Kin			
0.1±0.5	-	-	-
0.2±0.5	-	-	-
0.3±0.5	-	-	-
0.4±0.5	40	4.0±0.60	2.5±0.20
0.5±0.5	70	20.0±2.40	4.5±0.45

Variables given are mean±SE. NAA: Napthaleneacetic acid, BA: Benzylaminopurine, Kin: Kinetin, MS: Murashige and Skoog, SE: Standard error

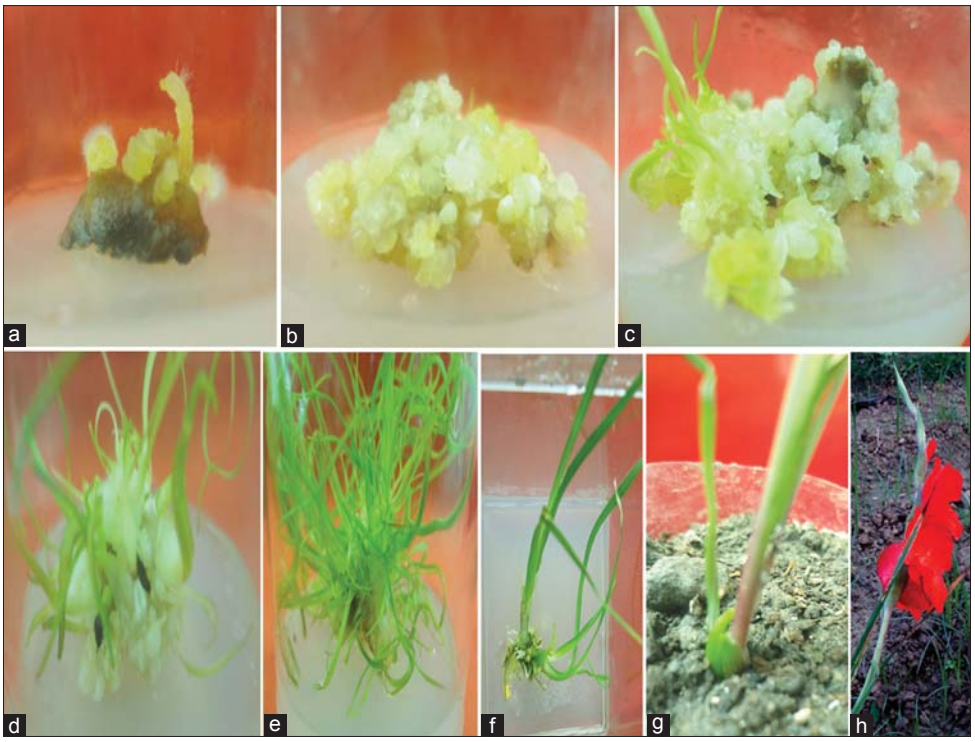

Figure 1: Induction of somatic embryos and regeneration into plantlets of locally cultivated orange color gladiolus (*Gladiolus dalenii*) through corm culture; (a) Corm explants proliferating embryogenic callus on Murashige and Skoog (MS) ± 7.5 mg/L napthaleneacetic acid culture after 30 days; (b) Highly proliferated embryogenic callus at the same medium culture after 90 days; (c) Prominent embryogenic callus with regeneration state on transferring to medium containing MS ± 0.5 mg/L benzylaminopurine ± 0.5 mg/L Kin culture after 30 days; (d). Prominent regeneration of embryogenic callus at the same medium transferring after 45 days; (e) Healthy and strong shoot formation at the same medium transferring after 60 days; (f) Root induction of somatic embryo-derived shoots on half-strength MS ± 2.5 mg/L indole-3-butyric acid culture after 30 days; (g) *In vitro* raised plant resumed new growth in the earthen-pot; (h) 4 months old *in vitro* raised plant at flowering state in the field

Table 3: Effects of IBA, IAA, and NAA on half-strength MS media in root induction from *in vitro* raised shoots at 30 days

Name of hormones	Concentrations (mg/l)	Percentage of shoot inducing roots	Average number of root-induced/shoot Mean±SE	Average root length (cm) Mean±SE
IBA	0.5	70	10.0±0.80	6.5±0.60
	1.0	50	8.0±0.60	6.0±0.20
	1.5	50	6.0±0.70	4.0±0.20
	2.0	50	7.0±0.40	4.5±0.30
	2.5	90	10.0±1.20	5.5±0.70
IAA	0.5	-	-	-
	1.0	10	5±0.30	4.5±0.70
	1.5	-	-	-
	2.0	-	-	-
	2.5	-	-	-
NAA	0.5	20	4.0±0.20	2.5±0.20
	1.0	30	4.0±0.60	2.5±0.40
	1.5	-	-	-
	2.0	-	-	-
	2.5	-	-	-

Variables given are mean±SE. NAA: Napthaleneacetic acid, IBA: Indole-3-butyric acid, IAA: Indole-3-acetic acid, Kin: Kinetin, MS: Murashige and Skoog, SE: Standard error

for maximum root induction. Shoots rooted well when they were excised individually and implanted on half-strength MS medium supplemented with 2.5 mg/L IBA, in which 90% shoot induced roots culture after 30 days. The average number of root per shoot was 10 ± 1.20, and the average root length of 5.5 ± 0.70 cm were observed in this medium (Figure 1f). The superiority of IBA for rooting over other auxins has also been reported (Amin and Akhter, 1983; Jaiswal and Amin; 1987; Amin *et al*., 1992; and Grewal *et al*., 1994).

Comparatively healthy rooted shoots were taken out from the culture vessels and washed gently under running tap water to get rid of agar. The *in vitro* rooted plantlets were then transferred to earthen pot (Figure 1g) containing a mixture of soil and compost (2:1) and covered with transparent polyethylene lid to maintain high humidity. After 1-week, the polyethylene lids were removed, and plantlets were kept in a shaded and misted twice a day. About 80% of the plantlets were resumed new growth within 30 days. A total number of 40 plantlets were survived in the field (Figure 1h) out of 50 *in vitro* regenerants.

CONCLUSION

From the above study, it can be concluded that among the plant growth regulators 7.5 mg/L NAA performed better for callus induction, combination of 0.5 mg/L BA ± 0.5 mg/L Kin showed excellent performance for

regeneration into shoots from the callus, and 2.5 mg/L IBA was found most responsive for root induction from callus derived shoots. Thus, the protocol developed in this study is useful for providing disease-free propagules for commercial cultivation, conservation, and international germplasm exchange whilst adequate planting material is a concern of this plant in the country.

REFERENCES

Aftab F, Alam M, Afrasiab H. *In vitro* shoot multiplication and callus induction in *Gladiolus* hybrids. Hortic Pak J Bot 2008;40:517-22.

Amin MN, Akhter S. Regeneration of plants *in vitro* from seedling explants of pomello (*Citrus grandis*). Plant Tissue Cult 1993;3:71-9.

Amin MN, Razzaque MA, Akhter S. Axillary shoot proliferation and adventitious rooting *in vitro* of carambola (*Averrhoa carambola* L.). Plant Tissue Cult 1992;2:7-13.

Begum S, Haddiuzaman S. *In vitro* rapid shoot proliferation and corm development in *Gladiolus grandiflorus* cv. Redbrand. Plant Tissue Cult 1995;5:7-12.

Blom BG, Aartrijk V. The regeneration of plants free of LSV and TBV from infected lilium bulb-scale explants in the presence of virazole. Acta Hortic 1985;164:163-8.

Bose TK, Yadav LP, Pal P, Das P, Parthasarathy VA. Commercial Flowers. 2nd rev ed., Vol. II. Bidhan Sarani, Kolkata: Naya Udyog; 2003.

Churrikova OA, Barykina RP. Regeneration ability of some bulbous and cormous monocotyledons in *in vitro* morphogenic aspect. Biologia 1995;50:50-5.

Dantu PK, Bhojwani SS. *In vitro* propagation of gladiolus: Optimization of conditions for shoot multiplication. J. Plant Biochem Biotech 1992;1:115-8.

Emek Y, Erdag B. *In vitro* propagation of *Gladiolus anatolicus* (Boiss.). Pak J Bot 2007;39:23-30.

Grewal HS, Dhatt AS, Gosal SS. Plantlet regeneration from callus in citrus. Plant Tissue Cult 1994;4:9-16.

Grewal MS, Arora JS, Gosal SS. Micropropagation of *Gladiolus* through *in vitro* cormel production. Plant Tissue Cult 1995;5:27-33.

Gupta R, Sehgal OP. Effect of media constituents on rooting of gladiolus shoot cultured *in vitro*. J Ornamental Hortic 1997;5:41-53.

Hussain I, Muhammad A, Rashid H, Quraishi A. *In vitro* multiplication of Gladiolus (*Gladiolus crassifolius*). Plant Tissue Cult 2001;11:121-6.

Iqbal MJ, Khan MM, Fatima B, Asif M, Abbas M. *In vitro* propagation of hybrid tea roses. Pak J Agric Sci 2003;40:155-63.

Jaiswal VS, Amin MN. *In vitro* propagation of guava from shoot culture of mature trees. J Plant Physiol 1987;130:7-12.

Kamo K, Chen J, Lawson R. The establishment of cell suspension cultures of Gladiolus that regenerate plants. *In vitro* Cell Dev Biol 1990;26:425-30.

Kamo K. Effect of phytohormones on plant regeneration from callus of Gladiolus cultivar 'Jenny Lee'. *In vitro* Cell Dev Biol 1994;30:26-31.

Kumar A, Sood A, Palni LM, Gupta AK. *In vitro* propagation of Gladiolus hybrids. Hort. Synergistic effect of heat shock and sucrose on morphogenesis. Plant Cell Tissue Organ Cult 1999;57:105-12.

Lilien KH, Kochba M. Mass propagation of Gladiolus hybrids. Acta Hortic 1987;212:631-8.

Logan AE, Zettler FW. Rapid *in vitro* propagation of virus indexed Gladioli. Acta Hortic 1985;164:169-80.

Misra S, Singh R. *In vitro* propagation of Gladiolus cv. Am Beauty J Ornam Hortic 1999;2:67-70.

Murashige T. Plant cell and organ culture as horticultural practices. Acta Hortic 1977;78:17-30.

Novak FJ, Petru E. Tissue culture propagation of *Lilium* hybrids. Sci Hortic 1981;14:191-9.

Pathania NS, Misra RI, Raghara SP. Precocious shoot proliferation and microcorm production in gladiolus through tissue culture. J Ornam Hortic 2001;4:69-73.

Prasad VS, Gupta SD. *In vitro* shoot regeneration of *Gladiolus* in semi-solid agar versus liquid cultures with support systems. Plant Cell Tissue Organ Cult 2006;87:263-71.

Priyakumari I, Sheela VL. Micropropagation of *Gladiolus* cv. 'Peach Blossom' through enhanced release of axillary buds. J Trop Agric 2005;43:47-50.

Rao TM, Negi SS, Swamy RD. Micropropagation of Gladiolus. Indian J Hortic 1991;48:171-6.

Roy PK, Kabir MH. *In vitro* propagation of sweet orange (*Citrus sinensis*) through plumule and cotyledon culture. Bangladesh J Life Sci 2006;18:107-12.

Sen J, Sen S. Two step bud culture technique for a high regeneration of Gladiolus corm. Sci Hortic 1995;64:133-8.

Singh AP, Dohare SR. Maximization of corms and cormel production in Gladiolus. In: Prakash J, Bhandary KR, editors. Floriculture-Technology, Trades and Trends. New Delhi: Oxford & IBH Pub., Co., Pvt., Ltd., India. 1994. p. 205-8.

Sinha P, Roy SK. Plant regeneration through *in vitro* cormel formation from callus culture of *Gladiolus primulinus* Baker. Plant Tissue Cult 2002;12:139-45.

Takagama S, Misawa M. A scheme for mass propagation of *Lilium in vitro*. Sci Hortic 1983;18:353-62.

Takagama S, Misawa M. Regeneration of organ formation by cytokinin and auxin in *Lilium bulbscales* grown *in vitro*. Plant Cell Physiol 1982;23:67-74.

Van AJ, Blom GJ, Linde CG. Lilies. In: Ammirato PV, Evans DA, Sharp WR, Bajaj YP, editors. Handbook of Plant Cell Culture. V5. London: Collier Macmillan Publishers; 1990. p. 535-76.

Ziv M, Halevy AH, Shilo R. Organ and plantlet regeneration of gladiolus through tissue culture. Ann Bot 1970;34:671-6.

Ziv M, Lilien HK. Gladiolus. In: Ammirato PV, Evans DA, Shark WR, Bajaj YP, editors. Handbook of Plant Cell Culture. New York: McGraw Hill Publishing Co.; 1990. p. 461-78.

Ziv M. The contribution of biotechnology to breeding, propagation and disease resistance in genotypes. Biotechnology and Propagation. In: Lilien HK, Halevy AH, Borochov A, editors. International Proceeding Int'l Symposiyam on Flower Bulbs. Acta Horticulture. 1997. p. 247-58.