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Research Article

POTENTIAL INFLUENCE OF CULTURE MEDIUM CONSISTENCE AND SILVER NITRATE ON MICROPROPAGATION OF *SOLANUM VIARUM,* AN ANTICANCER MEDICINAL PLANT

Sujana Papani[1*] and Naidu C.V[2]

1. Department of Botany & Biotechnology, P.V.K.N Government College, Chittoor, Andhra Pradesh.

2. Department of Biotechnology, Dravidian university, Kuppam, Andhra Pradesh.

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ABSTRACT

An understanding of optimal nutrient concentration could lead to increased growth and also could evoke maximum morphogenesis *in vitro* more effectively. Experiments were conducted to determine suitable combination of $AgNO_3$ concentration and artificial nutrient media to stimulate *in vitro* growth of *Solanum viarum*. All the four types of basal media such as Murashige and Skoog (MS), Schenk and Hilderbrandt (SH), Gamberg media (B_5) and K S medium were supplemented with 0 to 0.6 mg/L $AgNO_3$, 2mg/L BAP, 0.5mg/L IAA, 3% sucrose and 0.8% of agar. Leaf explants supplemented with M S media responded the best at 0.4 mg/L $AgNO_3$ with maximum mean shoot lengths (37.3 ± 0.46) and also with very high regeneration frequency (95%). Though maximum shoot lengths (10.2 ± 0.27 cm) were obtained in KS medium at 0.4mg/L $AgNO_3$, the plantlets were found to be fragile and chloretic and were found to be very less feasible in the subsequent cultures. Explant had shown minimum response in SH medium and also nil response was recorded in case of B_5 medium. So, MS media at 0.4 mg/L $AgNO_3$ concentration was found to be more consistent media for conserving and propagating Solanum *viarum* under *in vitro* conditions.

Key words: *Solanum,* Culture medium, Ethylene inhibitors, Shoot regeneration, Silver nitrate, Regeneration frequency

Abbreviations:

$AgNO_3$	Silver nitrate
MS	Murashige and Skoog
SH	Schenk and Hilderbrandt
B_5	Gamberg media
BAP	Benzyl Amino Purine
IAA	Indole Acetic Acid
Rf	Regeneration frequency

INTRODUCTION

Solanum viarum Dunal, belongs to the family Solanaceae, commonly called Tropical Soda Apple (TSA) is a shallow rooted, profusely branched, perennial shrub. It is native plant of Argentina and central Brazil (Mullahey *et al*, 1993) and has been naturalized in many parts of the world including India. Solasodine is a major glycoalkaloid constituent of this plant and it is a as the potential alternative for diosgenin which share a characteristic conversion to 16-dehydro pregnenolone acetate, the first step in steroid synthesis (Bhudavari, 1989). Solasodine extracted from this plant is used as a steroid precursor to produce contraceptive pills (Everist, 1981), treat cancer (Trouillas *et al*., 2005), Addison's disease, rheumatic arthritis, chronic asthma, leukemia (Blood cancer), obesity, palsy, skin diseases (Pingle and Dhyansagar, 1980), and anti-inflammatory steroidal drugs (Pandurangan *et al*., 2011). Despite economic importance, this plant is becoming endangered and threatened by over exploitation by pharmaceutical industries, urbanization, desertification, industrial development and attack by numerous parasites. So to overcome

**Corresponding author: Sujana Papani | E-mail: sujanapapani@gmail.com*

this problem and also in order to meet the world's pharmaceutical industries demand, there is a great need to establish an *in vitro* plant regeneration system to enhance their productivity and sustainability.

Micropropagation is one of the best alternatives to conserve medicinally important plants. The success of plant tissue culture is greatly influenced by several factors such as nature of tissue culture media, type of explants, *in vitro* environment and aseptic conditions. Growth and morphogenesis of plant tissue under *in vitro* condition are largely governed by the type and the consistency of the culture media. Culture media is nothing but artificially prepared nutrient media on which excised plant tissues and organs grow under *in vitro* conditions to multiply. Various culture media formulations proposed consists of several basic compounds which include mineral nutrients, an iron source, vitamins, amino acids, growth substances (hormones) and a carbohydrate supply.

Each plant has its own characteristic elementary composition which can be used to adapt the medium formulation. The suitable media result often in a much improved growth (Nas and Rea, 2004; Goncalves *et al.,*2005). The choice of culture medium usually depends on species to be cultured. Some species are sensitive to salts and some show better response on solid medium while others prefer liquid medium. Certain plant tissues are particularly sensitive to NH_4^+ (Ochatt and Caso, 1986) and Ochatt and power (1988a,b) found that protoplast of *pyrus* species would not tolerate this ion. Maintaing the the corrct balance of ions is very much essential in the media formulations. So, development of culture medium formulation for a particular plant species needs to be carefully drawn from systematic trial and experimentation considering the specific requirements of the plant. The total concentration of nitrogen in the media and the ratio of nitrate to ammonium ions, are the two important factors to be considered to find the suitable formulations of a media to different plant species. The formulations of the mineral components of the medium has been an empirical process since the beginning of tissue culture and MS (1962) media is most frequently used formulation for culturing many plant species.

Ethylene is a ubiquitous gaseous hormone which influences the growth as promoter or inhibitor based on the species used (Biddinton *et al.*, 1992). It is a simple hydrocarbon (C_2H_4), but has maximum impact on growth, cellular differentiation, fruit ripening and senescence in plants even at concentrations as low as 0.01µL L^{-1} or 10^{-6} % v/v (Reid, 1995). Ethylene production is directly regulated by internal signals and environmental stimuli from biotic and aboitic stress such as drought, chilling or freezing, hypoxia, water logging and pathogens attack (Wang *et al.*, 2002).Wounding during explants preparation, presence of high auxins and cytokinin concentration in media, further increases ethylene production in *in vitro* conditions. So, excess accumulation of gases directly influences the success of *in vitro* regeneration. Influence of ethylene in tissue culture propagation had been well documented previously. Ethylene suppresses the growth and morphogenesis of explants depending on the species and stage of the culture (Kumar *et al.*, 1998a).

Hence, in the present investigation, the consistency of nutrient composition in different media such as MS, B_5, KS and SH on *in vitro* morphogenesis and also antagonistic effect of various concentrations of $AgNO_3$ on ethylene action were studied to evolve highly reproducible *in vitro* protocol to conserve the medicinally important plant *Solanum viarum.*

MATERIAL AND METHODS

Plant material

Healthy seeds were pooled from ripen and dried fruits of *Solanum viarum* plant and were washed under running tap water for ten minutes to remove adherent fruit tissues and dried juice, to avoid fungal contamination while culturing. Then the seeds were soaked in 0.4mg/L (w/v) bavistin for one minute and 0.1% $HgCl_2$ for two minutes. Finally seeds were washed with double distilled water to remove the traces of all sterilants.

Under aseptic conditions seeds were inoculated on autoclaved MS media (Murashige and Skoog, 1962) basal medium. Prior to autoclaving at 121°C for 20 minutes, the pH of the medium was adjusted to 5.7 and cultures were maintained at 25 ± 2°C under 16 hrs and 8 hrs dark for 8 weeks. The primary shoots developed were further subcultured *in vitro* under aseptic culture conditions as mentioned above in 2 mg/L BAP supplemented MS media. Healthy and uniform leaves were excised from *in vitro* subcultured shoots and used as explants for further experiments.

Five replicates were maintained for all experiments and each experiment was carried out twice.

Culturing on various tissue culture media

Uniform sized leaves were inoculated to autoclaved different regeneration media like MS, B_5, SH and KS (table: 1), supplemented with 2mg/L BAP, 0.5 mg/L IAA and different concentrations of $AgNO_3$ ranging from 0 to 0.6 mg/L. All these different concentrations were maintained separately by following the same culture conditions of mother culture. Data of different variables such as regeneration percentage, total number of shoots per plant, shoot lengths were recorded from 5 replicates of each treatment after 6 weeks of observation.

RESULTS AND DISCUSSION

Effect of different concentration of $AgNO_3$ and various basal media on shoot regeneration from leaf explants

In the present investigation, the effect of different culture media formulations and various concentrations of silver nitrate along with plant growth regulators on *in vitro* morphogenesis was studied and observations recorded in table: 2. The results clearly indicate that, the explants have very poor regeneration frequency and shoot induction with unwanted callus formations in the SH and KS media and in B_5 media only very low callus formation without any shoot induction was recorded. Whereas explants cultured in MS media found to regenerate significantly with high mean number of shoots and mean shoot lengths in almost all concentrations of $AgNO_3$ tested with regeneration frequency ranging from 75 to 95%. Plants obtained in KS media at 0.4mg/L $AgNO_3$ concentration were found to be taller than (10.2 ± 0.27 cm) plantlets obtained in M S medium at 0.4 mg/L $AgNO_3$ (Figure:1). But, they look fragile and lightly chloretic.

Among different concentrations tested, MS media with 0.4mg/L $AgNO_3$ was found to be the best concentration to initiate 95% regeneration frequency with maximum mean number of shoots (37.3± 0.46) and mean shoot lengths (9.68 ± 0.10 cm). Consequently further regeneration studies were carried out in 0.4mg/L $AgNO_3$ in MS medium.

Composition of basal nutrient media always decides the successes of *in vitro* cultures. It is better to start with a known composition of nutrient media and modifications should be made accordingly to stimulate the maximum growth (Bhojwani and Razdan, 1983). Most of the plant species responded authoritatively in MS (Murashige and Skoog, 1962) nutrient media with slight modifications (Zhou *et al.*, 1994). The previous experiments clearly envisages, the fact that balance between ammonium ion and nitrate needs to be specially adjusted for each plant species, or that the total nitrogen content of the medium is the most important determinant of growth and morphogenesis (Phillips and Collins, 1979; Biedermann, 1987). Usually, in the medium presence of ammonium, in comparison with nitrate leads to rapid amino acid and protein synthesis at expense of carbohydrate synthesis. This diversion and cellular metabolism can contribute towards the formations of hyper hydric shoots and addition of nitrates can reverse this situation. Growth of plant cultures may also be impaired in media containing high concentration of NH_4^+ even when high concentrations of NO_3^- are present at the same time. Growth inhibition may not only be due to depressed pH (Mott *et al*., 1985) but also may reflect the toxicity induced by the accumulation of excess of ammonium ions. The free ammonium can cause toxicity, which at least in whole plants, can lead to an increase in ethylene evolution (Baker and Corney. 1987; Corney and Backa, 1987). That might be one of the major reasons for our plant *Solanum viarum* for showing nil response in B_5 medium, where ammonium sulphate has been used as the only source of the ammonium ion. Ammonium sulphate has not proved such a good source of NH_4^+ as ammonium nitrate or ammonium chloride (Singh, 1978; Kamada and Harada, 1979). Possibly the reason is that a medium containing ammonium sulphate has greater tendency to become more acidic (Harris.1956), than one containing less sulphate ions.

In our investigation, $AgNO_3$ at 0.4mg/L concentration in the MS media is best suitable for shoot induction. Various views and experimental evidences have been put forth to explain the silver ion's capability in blocking ethylene receptors (Bayer, 1976) to make plants insensitivity to ethylene. Silver ions are thought to perturb the ethylene ion binding site (Rodriguez et al., 1999). Binding process of ethylene to its binding site ETR 1 is mediated by Cu+ cofactors. The gradual replacement of copper cofactors with Ag+ brings about conformational changes in such way

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which continuously represses ethylene responses (Zhao et al., 2002).

CONCLUSION

Nevertheless media composition, sufficient quantity of ethylene inhibitors such as silver nitrate in the media also play vital role in engrossing the growth and morphogenesis of *Solanum viarum* under *in vitro* conditions. So, the totipotency of the cells would be expressed maximum to micropropagate, when the plant cell receives threshold level of mineral nutrients, vitamins, amino acids, carbohydrates besides, supplementation of exogenous and endogenous hormones. In our study, explants had not shown any organogenesis in B_5 medium which might be due to acidity (lowering of media pH) and also toxicity caused by ammonium sulphate constituency of that media. $AgNO_3$ at 0.4mg/L concentration in the MS media was found to be best option for micropropagation of *Solanum viarum.* After thorough review of literature, we understand that the effect of $AgNO_3$ concentration on various nutrient media was carried out for the first time. Further Molecular investigations should be carried out to understand the exact mechanism of silver antagonistic action on ethylene.

Table 1: Composition of various nutrient media used *in vitro* culture

Chemical constituents	MS medium	B_5 medium	SH medium	KS medium
1. Stock –A Macronutrients (10x) gm/500mL (FV 50 mL/L)				
NH_4N0_3	1650	---	---	7.24
$(NH_4)SO_4$	---	134	---	---
KNO_3	19.00	---	25	9.5
$MgSO_4.7\ H_20$	3.7	5	4	1.85
KH_2PO_4	1.0	30	---	0.68
$NH_4H_2PO_4$	---	---	---	3
2. Stock – B (100x) gm/100mL (FV 10 mL/L)				
$CaCl_2.2H_2O$	4.40	1.5	2	1.6
3) Stock-C Micronutrients (100x) mg/100mL (FV 1 mL/L)				
$MnSO_4.4H_2O$	2230	1000	1000	2500
$ZnSO_4.7H_2O$	860	200	100	1000
H_3BO_3	620	300	500	1000
Na_2MoO_4	25	25	10	25
$CuSO_4.5H_2O$	2.5	2.5	20	2.5
$CoCL_2.6H_2O$	2.5	2.5	10	--
4) Stock-D (100x) mg/100mL (FV 1 mL/L)				
KI	83	75	1000	100
5) Stock-E Iron stock (20x) mg/100mL (FV 5 mL/L)				
$FeSO_4.7H_2O$	557	280	280	280
$Na_2EDTA.2H_2O$	747	380	380	380
6) Stock – F Vitamin Stock (10x) mg/100mL (FV 2 mL/L)				

Nicotinic acid	25	100	25	250
Pyridoxine HCL	25	100	25	25
Thiamine HCL	10	1000	250	25
Folic acid				
7) Stock-G (10x) mg/100mL (FV 10 mL/L)				
Glycine	200	--	---	200
8) Stock-H (10x) mg/50mL (FV 5 mL/L)				
Myo-inositol	1000	10	10	1000
9) Solidifying agent mg/L				
Agar agar	8000	8000	8000	8000

Table 2: Effect of different tissue culture media and various concentrations of $AgNO_3$ on multiple shoot regeneration from leaf explants of *in vitro* grown *Solanum viarum* supplemented with 2.0 mg/L BAP and 0.5mg/L IAA.

Type of Medium	$AgNO_3$(mg/L)	Regeneration Frequency (%)	Mean number of shoots	Mean Shoot length (cm)	Callus formation
M.S	-	80	85± 0.3	3.98 ± 0.17	-
	0.1	85	20± 0.67	4.76 ± 0.07	-
	0.2	90	23 .7± 0.42	9.33 ± 006	-
	0.4	95	37.3± 0.46	9.68 ± 0.10	-
	0.6	75	8.7± .91	5.12 ± 0.03	-
B 5	-	-	-	-	-
	0.1	-	-	-	-
	0.2	-	-	-	+
	0.4	-	-	-	+
	0.6	-	-	-	+
SH	-		-	-	-
	0.1	20	1.2 ± 0.4	3.4 ± 0.76	-
	0.2	40	3.8 ± 0.45	3.8 ± 1.2	+
	0.4	-	-	-	++
	0.6	-	-	-	-
KS	-	-	-	-	-
	0.1	20	2.4 ± 1.2	2.2 ± 0.06	-
	0.2	40	3.4 ± 0.9	4.7 ± 0.34	+
	0.4	50	7.3 ± 1.1	10.2 ± 0.27	+
	0.6	10	1.2 ± 0.5	5.6 ± 0.6	++

Intensity of callus: + - very low, ++ - low

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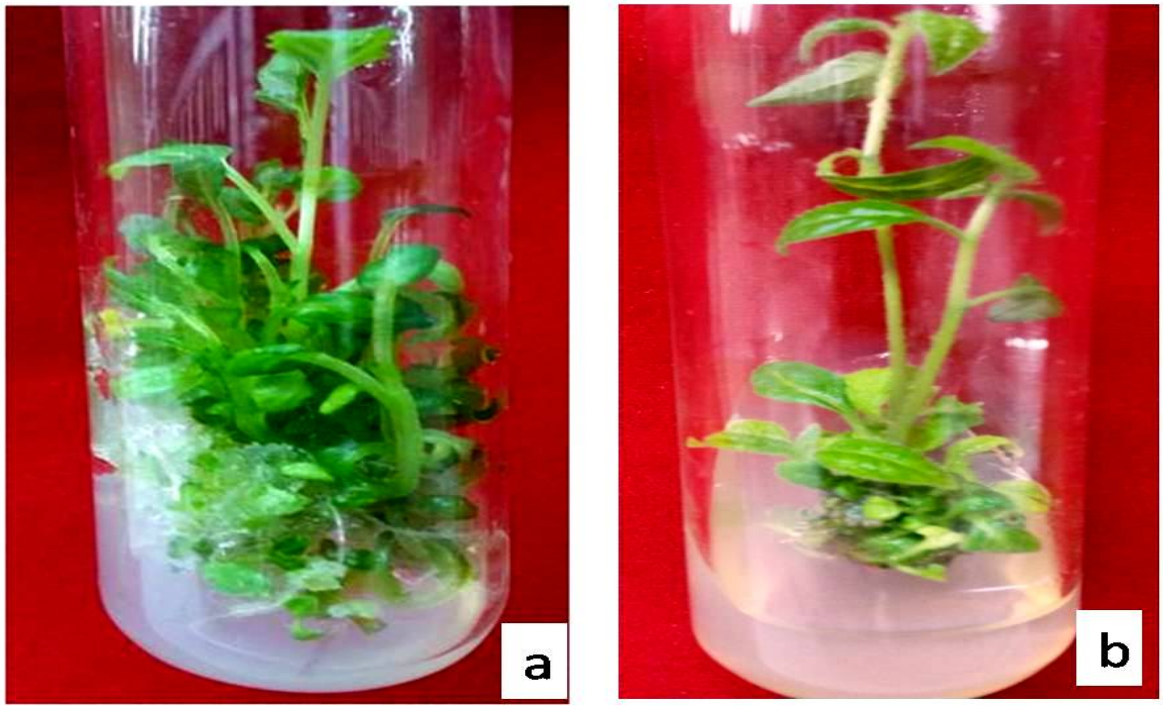

Figure 1: Effect of medium consistency on *in vitro* shoot organogenesis of *Solanum viarum* at various concentrations of $AgNO_3$.

a. **Multiple shoot formation from leaf explants on MS media supplemented with 0.4 mg/L $AgNO_3$ along with 2mg/L BAP and 0.1 mg/ IAA.**
b. **Multiple shoot formation from leaf explants on KS media supplemented with 0.4 mg/L $AgNO_3$ along with 2mg/L BAP and 0.1 mg/ IAA.**

REFERENCES

1. Babu, C.R. and Hepper, F.N. (1979). Taxonomy and nomenclature of *Solanum khasianum* and some of its relatives. Kew Bulletin, 34: 407 - 411.
2. Barker, A.V. and Corney, K.A. (1987). Ammonium-induced ethylene evolution by horticultural crops. Hortscience. 22: 381.
3. Bayer, E.M. (1976). A potent inhibitor of ethylene action in plants. J. Plant Physiol., 58: 268 – 271.
4. Bhojwani, S.S. and Razdan, M.K. (1983). Plant Tissue Culture. In: Theory and Practice. Elsevier Sci. Publ. Amesterdam., 194.
5. Biddington, N.L. (1992). The influence of ethylene in plant tissue culture. Plant Growth Regul., 11: 173 – 187.
6. Biedermann, I.E.G. (1987). Factors effecting establishment and development of *Mognolia* hybrids *in vitro.* Acta Horti., 212: 625 – 629.
7. Corney, K.A. and Berker, A.V. (1987). Physiology of ethylene evolution by tomato plants under ammonium induced stress. Hotriscience, 22: 381.
8. Everist, S.L. (1981). Poisonous plants of Australia. Angus and Robertson. ISBN 0-207-14228-9.
9. Goncalves, S., Correia, P.J., Martins-Loucao, M.A and Romano, A. (2005).A new medium formulation for *in vitro* rooting of carob tree based on leaf macronutrients concentration. Biol. Plant., 49: 277 - 280.
10. Harris, G.P. (1956). Amino acid as source of nitrogen for the growth of isolated oat embryos. New Phytol., 55: 253 – 268.
11. Kamada, H. And Harada, H. (1979). Studies on organogenesis in carrot tissue cultures. IIeffects of amino acids and inorganic nitrogenous compounds on somatic embryogenesis. Z. Pflangenphysiiol., 91: 453 – 463.
12. Kumar, P.P. Lakshmanan, P. and Thorpe, T.A. (1998a). Regulation of morphogenesis in plant tissue culture by ethylene. *In vitro* Cellular and Developmental Biology. Plant., 34: 94 - 103.
13. Mott, R.L, Cordts, J.M and Larson, A.M (1985). Nitrogen and growth regulator effects on shoot and root growth of soya bean *in vitro* pp. 336 – 337 in Henke, R.R., Hughes,K.W., Constantin, M.J., Hollander, A.A. and Wilson, C.M (eds). Tissue culture in forestry and agriculture.Plenum press, Newyork, London.

14. Mullahey, J.J. and Colvin, D.L. (1993). Tropical soda apple: A new noxious weed in Florida. Fact sheet WRS-7, University of Florida. Florida cooperative extension service.

15. Murashige,T.and Skoog, F.(1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plants., 15: 475 - 497.

16. Nas, M.N and Read ,P.E. (2004). A hypothesis for the development of a defined medium of higher plants and micropropagation of hazelnuts. Sci. Hortic.,101: 189 – 200.

17. Ochat, S.J. and Caso, O.H. (1986). Shoot regeneration from leaf mesophyll protoplasts of wild pear (*Pyrus communis*var. Pyraster L.). L. Plant. Physiol. 122: 243 – 249.

18. Ochat, S.J. andPower, J.B (1988a). Rhizogenesis in callus from conference pear (*Pyrus communis*var. L.) protoplasts. Plant Cell Tissue Organ Cult., 13: 159 – 164.

19. Pandurangan, A., Khosa, R.L. and Hemalatha, S. (2011). Anti-inflammatory activity of an alkaloid from Solanum trilobatum on acute and chronic inflammation models. Nat. Prod. Res., 25: 1132 - 1141

20. Phillips, G.C. and Collins, G.B.(1979). *In vitro* Tissue culture of selected legumes and plant regeneration fromcallus cultures ofred clover. Crop.Sci., 19: 59 – 64.

21. Pingle, A.R. and Dhyansagar, V.R. 1980 *Solanum viarum* as a source of solasodine. Indian Drugs, 17: 366 - 370.

22. Reid, M.S. (1995). Ethylene in plant growth, development and senescence. In: Davies, P.J. editor. Plant Hormones. Dordrecht, Kluwer Academic Publishers, The Netherlands, 486–508.

23. Rodriguez, F.I., Esch, J.J., Hall, A.E., Binder, B.M., Schaller, G.E. and Bleecker, A.B. (1999). A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis.* Science, 283: 996 - 998.

24. Singh, J.P. (1978). Effect of nitrogen sources on shoot bud differenciation of *Dioscorea deltoidea* Wall. Callus culture. Biol. Plant., 20: 436- 439.

25. Trouillas, P., Corbière, C., Liagre, B., Duroux, J.L. and Beneytout, J.L. (2005). Structure-function relationship for saponin effects on cell cycle arrest and apoptosis in the human 1547 osteosarcoma cells: a molecular modeling approach of natural molecules structurally close to diosgenin. Bioorg. Med. Chem., 13: 1141 - 1149.

26. Wang, K.L.C., Hai, L. and Ecker, J.R. (2002). Ethylene biosynthesis and signaling networks. Plant Cell, S131-S151.

27. Zhao, X.C., Qu, X., Mathews, D.E. and Schaller, G.E. (2002). Effect of ethylene-pathway mutations upon expression of the ethylene receptor ETR1 from *Arabidopsis*. Plant Physiology, 130: 1983 - 1991.

28. Zhou, J., Hui, M., Guo, F. and Luo, X. (1994). Effect of thidiazuron on somatic embryogenesis of *Cayratia japonica.* Plant Cell Tiss. Org. Cult., 36: 73 – 79.

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