GEOBIOS 1 : 160—164, 1974

GROWTH OF *ZIZYPHUS JUJUBA* LAMK. GALL AND STEM TISSUES IN CULTURE WITH NITROGEN SOURCES

G.S. VYAS*, P. TANDON AND H C. ARYA

Department of Botany, University of Jodhpur, Jodhpur—342001, India
(Received August 8; Revised September 11, 1974)

SUMMARY

Normal tissue of *Zizyphus jujuba* grew well on modified Murashige and Skoog's (MS, 1962) medium with potassium or calcium nitrate (1.9g/l) only, but gall tissue grew best on MS medium containing both KNO_3 (1.9g/l) and NH_4NO_3 (1.65g/l). Growth of tissues was poor on MS medium with all other ammonium salts as sole source of nitrogen. None of the organic sources of nitrogen could serve as sole source of nitrogen except urea (0.5g/l). Addition of DL-α alanine and DL-β phenylalanine to the medium containing usual nitrates enhanced the growth of both the tissues. But L-glutamic acid along with nitrates increased the growth of gall tissue only. Glutathione, DL-methionine and L-cystine inhibited the growth of both the tissues.

INTRODUCTION

The nutritional requirement of different plant tissues varies. Importance of different organic and inorganic sources of nitrogen for plant growth in general has been reviewed (White, 1943; Burström, 1945; Frank et al., 1951). Urea served as sole source of nitrogen while alanine, glycine, arginine, asparagine and glutamic acid did so partially in case of sunflower crown-gall tissue (Riker and Gutsche, 1948); in tobacco tumor tissue (Frank et al., 1951); in virus tumor tissue of *Rumex acetosa* (Nickell and Burkholder, 1950). Paris and Duhamet (1953) found a mixture of amino acids that increased the growth of *Scorzonera* crown-gall tissue to the same extent as did the whole coconut milk. In case of sunflower crown-gall tissue Eberts et al. (1954) found that tissues contained active glutamic acid, glutaric acid, and alanine transaminases. They also recorded that nitrates had no effect on ammonia uptake and utilization of certain amino acids along with nitrate.

In certain cases organic nitrogen along with inorganic nitrate was necessary for balanced growth of plant tissues. Beneficial effects of amino acids of casein hydrolysate for carrot tissue cultures (Blakeley and Steward, 1961) and a mixture of amino acids for tobacco tissue cultures (Braun, 1958) are reported. Studies have also been made on growth promoting activities of certain selected amino acids (Steward et al., 1958; Street et al., 1961, and Sutton et al., 1961).

In case of plant gall tissues induced by insects and mites, nitrogen nutrition has been studied in greater detail with *Phylloxera* gall and normal grape stem tissues (Hildebrandt, 1963; Arya, 1965). Adenine sulphate in various combinations with casein hydrolysate inhibited growth of gall friable tissue type (Pelet et al., 1960). Hildebrandt (1963) reported in brief that gall and normal tissue clones grew poorly on mineral salt sucrose medium supplemented with various combinations of eighteen amino acids, alpha-naphthalene acetic acid, adenine and kinetin. Arya (1965) reported better growth of gall tissue clones as compared to normal tissue clones on pancreatic digest of casein hydrolysate medium. In the present investigation nitrogen requirement of gall and normal stem tissues of *Zizyphus jujuba* has been studied.

MATERIALS AND METHODS

Normal callus tissue from *Z. jujuba* hypocotyl and tissue from galls incited by *Eriophyes cernuus* Massee,, were isolated on MS medium. Normal callus tissue was shining white, compact, hard and very sensitive to injury. Necrosis occurred quite frequently especially in its inner part. Necrotic tissues were carefully removed before transferring the tissue

*Present address : Department of Botany, Govt. College, Damoh, M.P.

explants to the medium. Gall-callus tissue was light yellow in colour and friable.

To make transfers, a 3 week old tissue was removed from stock cultures to a petriplate and was cut into pieces aseptically. Two pieces each weighing 100 mg by fresh weight were placed on agar nutrient medium. Forty ml. of medium was taken in each conical flask of 100 ml. capacity and plugged with non absorbant cotton. The medium was adjusted to pH 5.8 before autoclaving. The cultures were grown under florescent light of 1000 lux intensity at a temperature of 26±2° and 60% relative humidity.

Six inorganic nitrogen compounds viz. sodium nitrate, potassium nitrate, calcium nitrate, ammonium sulphate, and ammonium chloride and six organic nitrogen compounds viz. urea DL-α alanine, DL-β phenylalanine, DL-aspartic acid, glycine and L-glutamic acid were used as sole source of nitrogen. All these amino acids plus glutathione, DL-methionine and L-cystine were used along with usual nitrates.

The tissues were grown on MS medium with or without KNO_3 (1.9 g/l) and NH_4NO_3 (1.65 g/l) but supplemented with various nitrogen compounds. Glycine was omitted and KNO_3 was replaced by equimolar quantity of KCl in MS medium to study the effects of various nitrogen sources. Concentrations used were 0.1g/l to 4.0g/l for all the inorganic nitrogen compounds and urea. Amino acids were used in concentrations of 0.05g/l to 0.4g/l either singly or in combination with nitrates. The tissues grown on MS medium without any modifications were used as control. To compare the results, the tissues were also grown in MS medium without nitrate and glycine.

All exogenous supplied nitrogenous compounds except urea wereautoclaved with themedium. Urea being thermolabile was sterillized at room temperature using bacteriological filter and added by burette to autoclaved medium just before solidification.

Average wet weight of ten replicates after thirty days of tissue growth was used as a measure of growth. Growth was determined by an approximate comparison of wet weight with that of control. It is indicated as follows: 0=none, Trace=growth less than 1/4 of the control, Poor=growth 1/4 of the control, Fair =growth 1/2 of the control, Good=growth 3/4 of the control, Excellent=growth equal to or greater than control.

RESULTS

Inorganic sources of nitrogen : Effects of different concentrations from 0.1 to 4.0g/l of different inorganic sources of nitrogen on growth of normal and gall tissues are presented in Figs. 1 and 2. From these figures it is evident that normal tissue growth was excellent with $Ca(NO_3)_2$ and KNO_3, good with $NaNo_3$ and NH_4NO_3, and poor with $(NH_4)_2SO_4$ and NH_4Cl as sole source of nitrogen in the medium The optimal was 2.0g/l with nitrate salts and 0.5g/l. with ammonium salts. Growth remained steady with 4.0g/l of $Ca(NO_3)_2$, KNO_3 and $NaNO_3$. However, it was reduced with 2.0g/l and 4.0g/l of $(NH_4)_2SO_4$ and NH_4Cl and 4.0g/l of NH_4NO_3. Results given in Fig. 2 showed that none of the inorganic nitrates used singly as a sole source of nitrogen supported excellent growth of gall tissue. However, gall tissue growth was good with NH_4NO_3 or $Ca(NO_3)_2$ fair with $NaNO_3$ or KNO_3 and poor with $(NH_4)_2SO_4$ and NH_4Cl. The optimal for gall tissue growth was 0.5g/l with all the inorganic nitrates used except NH_4NO_3. 2.0g/l of NH_4NO_3 was optimal for gall tissue growth. Concentrations above 0.5g/l of $NaNO_3$, KNO_3, $Ca(NO_3)_2$, $(NH_4)_2SO_4$ and NH_4Cl and 4·0g/l of NH_4NO_3 were inhibitory for gall tissue growth.

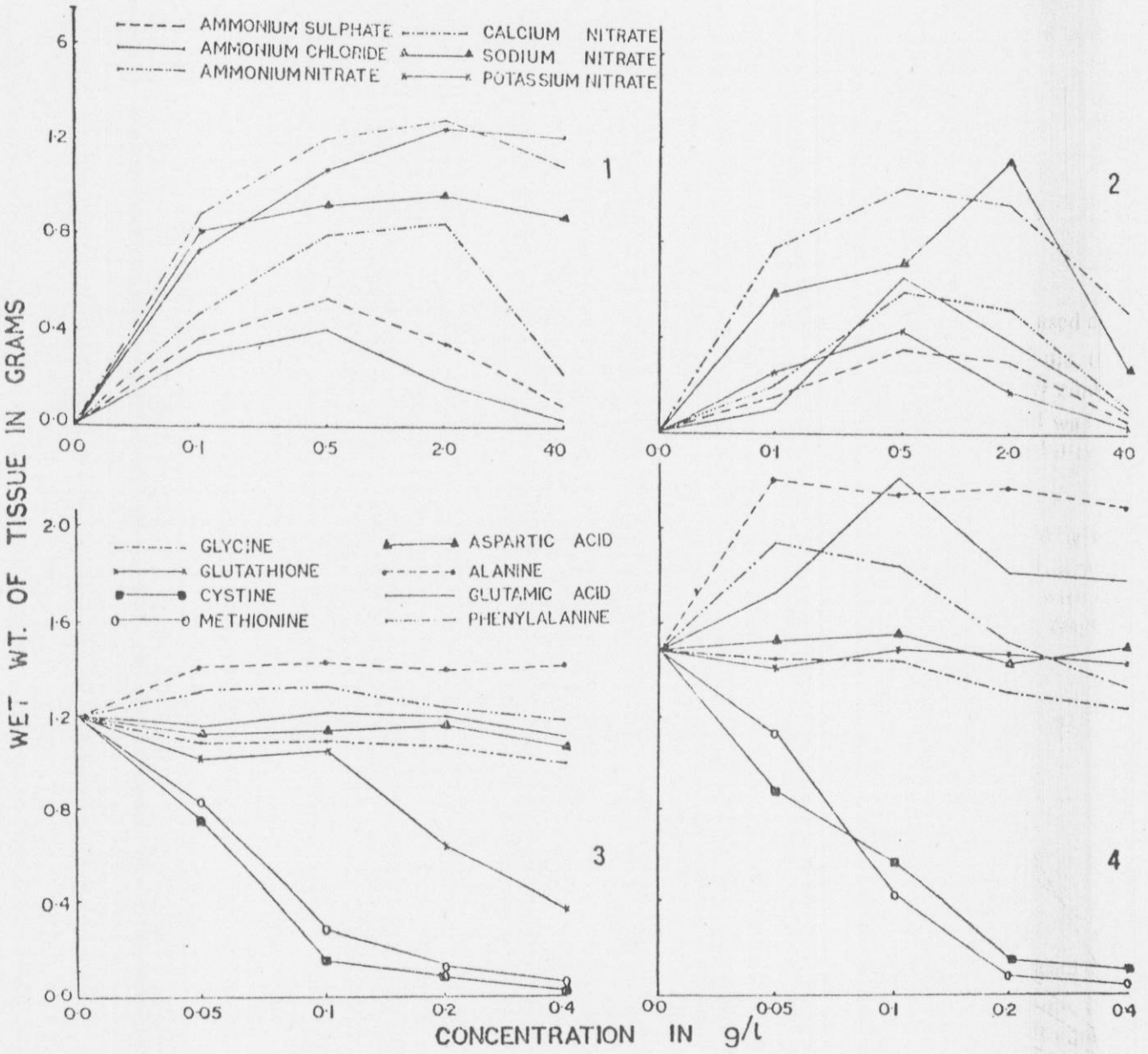
Organic sources of nitrogen : Different concentrations ranging from 0.1g to 4.0g/l of urea and 0.05 to 0.4g/l of DL-α alanine, DL-β phenylalanine, DL-aspartic acid, L-glutamic acid and glycine were used to study their effects. The results obtained are given in the Table 1. It is evident from the data that none of the organic nitrogen sources tested served as solo sources of nitrogen for growth of both normal and gall tissues. However, urea (0.5g/l) yielded 620 mg of gall tissue and 480 mg of normal tissue. L-Glutamic acid (0.4g/l) yielded 480 mg of normal tissue in the first passage which was subsequently reduced to 260 mg in successive passages. Similarly DL-α alanine (0.4g/l) yielded 680 mg of gall tissue in the first passage but 280 mg in subsequent passages.

Amino acids in combination with nitrates : Effect of addition of 0.05g/l to 0.4g/l of DL-α alanine, DL-β

phenylalanine, DL aspartic acid, L-glutamic acid, glycine, glutathione, L-cystine and DL-methionine in MS medium containing nitrates was studied. Results obtained are given in Fig. 3 and 4.

Normal tissue growth : Results given in Fig. 3 showed that addition of DL-α alanine, DL-β phenylalanine increased growth of tissue. Tissue yield remained unaffected with the addition of L-glutamic acid, glycine and DL-aspartic acid in the medium. DL-Methionine and L-cystine inhibited tissue growth progressively with their respective increased concentrations in the medium. With glutathione (0·1g/l) growth remained unaffected but its higher concentrations (0·2g/l to 0·4g/l) inhibited growth. It was important to note that glutathione induced friability in the texture of the tissue.

Gall tissue growth : Results obtained with gall tissue growth (Fig. 4) indicated that DL-α alanine, DL-β phenylalnine and L-glutamic acid with nitrate in the medium increased growth of tissues, respectively.



Figs. 1 - 4. Effect of inorganic sources of nitrogen on normal tissue (1) and gall tissue (2). Effect of amino acids plus nitrates on normal tissue (3) and gall tissue (4).

Growth remained unaffected with the addition of glycine and DL-aspartic acid in the medium. L-Cystine and methionine inhibited tissue growth progressively as concentrations increased. Glutathione (0.2 to 0.4g/l) was inhibitory to tissue growth.

Table 1. Comparative growth of normal and gall tissue on MS medium supplemented with some organic nitrogenous compounds as sole source of nitrogen.

Nitrogen source	Conc. in g/l	Average wet wt. in mg. Normal	Gall
Urea	0.1	210	460
	0.5	620	480
	2.0	180	124
	4.0	150	120
Aspartic acid	0.05	110	140
	0.1	120	125
	0.2	160	130
	0.4	148	136
Alanine	0.05	113	118
	0.1	126	190
	0.2	147	274
	0.4	168	280
Phenylalanine	0.05	114	110
	0.1	119	116
	0.2	144	156
	0.4	136	128
Glutamic acid	0.05	150	126
	0.1	248	134
	0.2	256	118
	0.4	260	127
Glycine	0.05	144	135
	0.1	120	143
	0.2	178	154
	0.4	150	134
Nitrogen deficient media		110	124

CONCLUSION

It may be concluded from the results obtained that nitrate was superior to ammonium salts as sole source of nitrogen for the growth of both the tissues. Normal tissue grew better in MS medium with potassium nitrate or calcium nitrate as sole source than control with usual nitrates, whereas the gall tissue responded poorly. The manner in which the ammonium salts along with usual nitrates proved better for growth of diseased tissue is not clear. It appears, therefore, logical to assume that rapidly growing gall tissue may require some reduced form of nitrogen in the form of ammonium ions in addition to nitrate to meet the nitrogen requirement.

Among organic sources urea (0.5g/l) could partially support growth of both the normal and gall tissues. Normal tissue with L-glutamic acid (0.4g/l) and gall tissue with DL-α alanine (0.4g/l) as sole source of nitrogen could grow in first passage but in subsequent passages growth of normal and gall tissue could not be sustained. However, with other amino acids used as sole source of nitrogen both the tissues failed to grow in first passage itself.

Incorporation of DL-α alanine and DL β phenylalanine in the medium containing unusual nitrate increased the growth of both the tissues but L glutamic acid with nitrate proved beneficial for gall tissue only. All the sulphur containing amino acids used with usual nitrates inhibited growth of both the tissues.

REFERENCES

Arya, H.C., Cultural behaviour of insect gall and normal plant stem single cell clones. 293-304. *In Tissue Culture* ed. C.V. Ramakrishnan, Dr. W. Junk Publishers, The Hague, 1965.

Blakeley, L.M. and Steward, F.C., Growth induction in cultures of *Haplopappus gracilis*. I. The behaviour of cultured cells. *Amer. J. Bot.*, 1961, 48, 351-358.

Braun, A. C., A physiological basis of autonomous growth of crown-gall tumor cell. *Proc. Natl. Acad Sci.*, 1958, 44, 344-349.

Burstrom, H., The nitrate nutrition of plants. A general survey of occurence and assimilation of nitrate. *Ann. Royal Agric. Coll. Sweden*, 1945, 13, 1-86.

Eberts, F.C. Jr., Burris, R.H., and Riker, A.J., The metabolism of nitrogenous compounds by sunflower crown gall tissue cultures. *Pl. Physiol.*, 1954, 29, 1-10.

Franz, E.M., Riker, A.J. and Dye, S.L., Comparison of growth by tobacco and sunflower tissue on synthetic media containing various sources of organic nitrogen. *Pl. Physiol.*, 1951, 26, 258-267.

Hildebrandt, A.C., Growth of single cell clones of diseased and normal tissue origins. 1-20. *In Pl. Tissue Culture and Morphogenesis*, ed. J.C.O. Kelley, Scholars Library, N.Y., 1963.

Murashige, T, and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 1962, 15, 473-497.

Nickel, L.G., and Bulkholder, P.R., A typical growth of plants. II. Growth *in vitro* of virus tumors of *Rumex* in relation to temperature, pH, and various sources of nitrogen, carbon and sulphur. *Amer. J. Bot.*, 1950, 37, 538–547.

Paris, D., and Duhamet, L., Action d[1] un me lange d[1] acids amines et de vitamines sur la proliferation des cultures de tissue the crown gall de *Scorzonera* comparison avec l action due lait de coco , *Comdt. Rend. Acad. Sci.*, 1953, 236, 1690–1692.

Pelet, F., Hildebrandt, A.C., Riker, A.J., and Skoog, F., Growth *in vitro* of tissue isolated from normal stem and insect galls *Amer. J. Bot.*, 1960, 47, 186–195.

Riker, A.J., and Gutsche, A E., The growth of sunflower tissue *in vitro* on synthetic media with various organic and inorganic sources of nitrogen. *Amer. J. Bot.*, 1948, 35, 227–238.

Steward, F.C., Pollard, J.K., Patchett, A.A., and Witkop, B., The effects of selected nitrogen compounds on the growth of plant tissue cultures. *Biochem. et. Biophys. Acta.*, 1958, 28, 308–317.

Street, H.E., Garter, J.E., Scott, E.G. and Sutton, D., Studies on the growth In culture of excised wheat roots. I. The growth effects of an acid hydrolysed casein and of light. *Phsiol. Plan.*, 1961, 14, 621-631.

Svtton, D., Scott, E.G. and Street, H.E., Studies on growth in culture of excised wheat roots. II The growth promoting activity of amino acids., *Physiol. Plant.*, 1961, 14, 712–724.

White, P.R., *A Handbook of Plant Tissue Culture.* The Jaque cattel press, Lancaster, Pa., 1943.