FACTORS AFFECTING PLANT RECOVERY OF CULTURED EMBRYOS OF THREE *GLYCINE* GENOTYPES

by

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A very special thank you goes to my daughters, Adrienne and Jessica. Due to their support, understanding and love, Mom reached her goal.

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LITERATURE REVIEW

The genus *Glycine* Willd., as currently delimited, consists of two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The cultivated soybean, *Glycine max* (L.) Merr., and the "wild soybean", *G. soja* Sieb. & Zucc., constitute the subgenus *Soja*. Both species are annuals with a diploid number of 2n=40 (HADLEY & HYMOWITZ, 1973). Hybrids between *G. max* and *G. soja* can be readily obtained, and the two species appear to be genetically compatible (AHMAD et al., 1977; NEWELL, 1986; Twelve species are proposed as belonging to the subgenus *Glycine*, which is undergoing taxonomic revision (HYMOWITZ, 1987; NEWELL, 1986). *Glycine arenaria* Tind., *G. argyrea* Tind., *G. canescens* F.J. Herm., *G. clandestina* Wendl., *G. curvata* Tind., *G. cyrtoloba* Tind., *G. falcata* Benth., *G. latifolia* (Benth.) Newell and Hymowitz, *G. latrobeana* Benth. and *G. microphyla* Tind. are indigenous to Australia and are diploid (2n=40). Predominantly tetraploid (2n=80), *G. tabacina* (Labill.) Benth. is also found in Australia as well as in Taiwan, the West Central and South Pacific Islands and South China. Occasional diploid forms of *G. tabacina* are also found. *Glycine tomentella* Hayata is found in tetraploid (2n=80) forms in Taiwan, the south coast of China and the northern Philippines. Diploid, tetraploid and aneuploid (2n=38,78) forms are found in Australia (HYMOWITZ, 1987; NEWELL, 1986; SINGH & HYMOWITZ, 1985a; SINGH & HYMOWITZ, 1985d).

All of the *Glycine* species are perennial, twining or scrambling herbs with a thick tap root. These plants grow in such diverse environments as arid zones, alpine regions and tropical areas (BROUÉ et al., 1982; BURDON & MARSHALL, 1981b). Physiological traits possessed by the wild species include drought, heat and cold tolerance; daylength neutrality and salt tolerance (BROUÉ et al., 1982; HYMOWITZ, 1986; NEWELL & HYMOWITZ, 1982). Screening of the perennials has shown them to carry resistance for leaf rust, *Phakopsora pachyrhizi* Syd., yellows mosaic virus, *Phaseolus virus* 2 and powdery mildew, *Erysiphe polygoni* D.C. (BURDON & MARSHALL, 1981a; BURDON & MARSHALL, 1981b; NEWELL AND HYMOWITZ, 1982; SHANMUGASUNDARAM, 1986). Accessions among several species have also demonstrated resistance to races 3 and 4 of *Hederodera glycines* Ichinohe, the soybean cyst nematode, as well as tolerance to brown spot, *Septoria glycines* Hemmi (HYMOWITZ, 1986; RIGGS, 1986).

Perennial *Glycine* species, possessing the above mentioned characters, represent a potentially valuable source of genetic diversity for improvement of cultivated soybeans (BROUÉ et al., 1982). Genetic variability in *G. max* is limited to introductions of germplasm from Korea, Japan and the People's Republic of China. While China is considered to be the primary gene center for soybean, germplasm has not been collected in any appreciable quantity from this source since 1931 (HYMOWITZ & NEWELL, 1980). SCHOENER &

FEHR (1979) and DELANNAY et al. (1983) have addressed the problem of genetic uniformity in the gene pool of North American soybean cultivars. Both report that the genetic base of modern cultivars can be traced to about 12 ancestors for nuclear genes, while only five plant introductions account for the cytoplasm of 121 northern cultivars. Wild relatives of cultivated species have been used effectively for the improvement of crop plants in numerous breeding programs (BROWN, 1982; HARLAN, 1976; SHARMA & GILL, 1983; STALKER, 1980). Attempts to improve the soybean through interspecific hybridization with the wild species of the subgenera *Glycine* have been hampered by the confusion which has surrounded the genus *Glycine* Willd. since its inception (HERMANN, 1962).

In the 1753 edition of *Species Plantarum*, Linnaeus assigned eight species to the genus *Glycine*. All of these have since been transferred to other genera. The cultivated soybean was listed in the same publication both as *Phaseolus max* L. and as *Dolichos soja* L. (HERMANN, 1962). General acceptance of the combination *Glycine max* (L.) Merr., proposed by Merrill in 1917, ended the controversy surrounding the proper nomenclature of the soybean (LAWRENCE, 1949; PACLT, 1949; PIPER, 1914; PIPER & MORSE, 1923; RICKER & MORSE, 1948).

The practice of early taxonomnists to assign all *Phaseoleae* which lacked differentiating characters to the gen-

era *Glycine* and *Dolichos* led to such a vague definition of the genus *Glycine* that, at one point, 286 species were listed in *Index Kewensis*. Additional reports of subspecies and varieties increased the number to 326 (NEWELL & HYMOWITZ, 1978). HERMANN'S (1962) major revision of the genus *Glycine* and its allies in 1962 provided the first usable system of classification. The genus was further clarified by VERCOURT (1966), who suggested that the closest wild relative of the soybean be referred to as *G. soja* Sieb. & Zucc. rather than the commonly used *G. ussuriensis* Regel & Maack.

Lack of experimental material hindered initial efforts to establish relationships among the members of the subgenus *Glycine* (NEWELL & HYMOWITZ, 1983). The expansion of the perennial germplasm collection, through collecting efforts by researchers in the United States and Australia, has resulted in progress in both interspecific hybridization and classification of the *Glycine* species (NEWELL & HYMOWITZ, 1983). Cytogenetic analysis of both intra- and interspecific hybrids has helped to establish phylogenetic relationships among the wild *Glycine* species (GRANT, 1984; GRANT et al., 1984; NEWELL & HYMOWITZ, 1983; SINGH & HYMOWITZ, 1985a,b,c,d; TINDALE, 1984). These studies indicate that the closeness of the parental genome influences the crossability, hybrid seed viability and seed fertility of F_1 hybrid plants.

In 1983, NEWELL & HYMOWITZ identified *G. latifolia* (Benth.) Newell and Hymowitz as a species separate from *G. tabacina* and *G. tomentella*, with which it had been confused. TINDALE (1984) removed the curved pod forms from *G. clandestina* to establish *G. cyrtoloba* as a separate species. She also added *G. argyrea* to the subgenus as a previously undescribed taxon (SINGH & HYMOWITZ, 1985d; TINDALE, 1984). Based upon recent collections and cytogenetic studies, it has been proposed that three additional species be added to the subgenus *Glycine*. These are: *G. arenaria* Tind., *G. curvata* Tind. and *G. microphyla* Tind. (HYMOWITZ, 1987).

Successful hybridization within the subgenus *Glycine* was first reported by PALMER & HADLEY in 1968. Reciprocal interspecific hybrids were obtained between *G. tabacina* and *G. latifolia*, reported as *G. tomentosa* by the authors (NEWELL & HYMOWITZ, 1980 ,1983). The F_1 hybrids obtained were highly male and female sterile (PALMER & HADLEY, 1968). These authors also mention unsucessful attempts to cross these two perennial accessions with *G. max*. In 1979, BROUÉ et al. and PUTIEVSKY & BROUÉ reported obtaining two intraspecific and six interspecific F_1 plants. Three additional interspecific hybrid combinations were obtained by NEWELL & HYMOWITZ in 1983. All of the above mentioned hybrids were obtained using traditional crossing methods.

Attempts to hybridize *G. max* with the wild perennials

using standard methods resulted in small pods that turned yellow and abcissed 10 to 21 days after pollination (HOOD & ALLEN, 1980; LADZINSKY et al., 1979; NEWELL & HYMOWITZ, 1983). The same phenomenon occurred for certain crosses among perennial species (GRANT et al., 1984). Success rates of crossing ranged from 1-56% within species and from 5-17% among species, indicating incompatibilities between species and among genotypes of the same species (BROUÉ et al., 1979; NEWELL, 1986; NEWELL & HYMOWITZ, 1982; PUTIEVSKY & BROUÉ, 1979). Workers reasoned that since pod formation was initiated following fertilization, cessation of endosperm development was a probable cause for pod abortion (LADZINSKY et al., 1979; NEWELL & HYMOWITZ, 1982). Embryo or ovule culture was seen as a method for overcoming this barrier. Immature soybean embryos previously had been raised to maturity in culture (CUTTER & BINGHAM, 1975).

The first hybrid between the soybean and a wild, perennial relative was reported by NEWELL & HYMOWITZ in 1982. Culturing ovules in a modified liquid version of GAMBORGS et al.'s (1968) B-5 medium, they recovered seven, viable hybrid plants. Six of these were from *G. max* cv. Altona x *G. tomentella* IL 428 (University of Illinois No.) (2n=78) and one from *G. max* cv. Altona x *G. tomentella* P.I. 441.002 (2n=80). That same year, BROUÉ et al. reported the recovery of five interspecific hybrid plants resulting from a cross between a synthetic amphiploid of ((*G. tomentella*

(1316) x *G. canescens* (1232)) x *G. max* cv. Lincoln. Both ovules and embryos were cultured using the "nurse endosperm" technique developed by DELATOUR et al.(1978) and WILLIAMS' (1978) medium. Subsequent hybridizations, both among perennial species and between perennial *Glycine* accessions and *G. max*, have been reported by GRANT et al.(1984) and by SINGH & HYMOWITZ (1985d). Additional hybrid combinations have been obtained between *G. max* and *G. canescens* and between *G. max* and an F_1 (*G. argyrea* x *G. canescens*) (NEWELL, 1986).

All hybrids between *G. max* and perennial species have been reported to be sterile. The chromosome number of several of the F_1 hybrids has been doubled with colchicine as a means to overcome sterility. To date, a low level of fertility has been restored to a *G. max* x *G. tomentella* hybrid (HYMOWITZ & SINGH, 1984; NEWELL, 1986).

Several researchers have indicated that sucrose level, salts concentration and plant hormone interactions in embryo culture media are interrelated and highly variable according to plant species and developmental stage of the embryo being cultured (AMMIRATO, 1977; NORSTAG, 1979; RAGAHAVEN & TORREY, 1963). Immature hybrid soybean embryos and ovules have been successfully cultured using various media, with rescue success ranging from 1 to 4% (NEWELL, 1986). Both NEWELL & HYMOWITZ (1982) and SINGH & HYMOWITZ (1985d) used GAMBORG et al.'s (1968) B-5 medium supplemented with

glycine and 100 g/l sucrose. The medium also contained 6-benzylaminopurine and 6-furfurylaminopurine at 1 mg/l and 0.64 mg/l, respectively. BROUÉ et al. (1982) used WILLIAMS' (1978) medium, which has a low salts concentration, a high level of vitamins, sucrose at 15 g/l and no hormones. GRANT et al.,(1984) cultured embryos and ovules on several media types, including that used by BROUÉ in 1982. An artificial medium developed by BOURGIN & NITSCH (1967) was also used, as was a version of MURASHIGE & SKOOG'S (1968) medium, modified by the addition of minor salts at ten times the reported rate and the addition of 1-naphthalene acetic acid and zeatin at the rate of 0.018 mg/l and 2.19 mg/l, respectively. Sucrose was included in all media types used by GRANT at the rate of 30 g/l.

INTRODUCTION

The genus *Glycine* Willd., as currently delimited, consists of two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm.. The cultivated soybean, *Glycine max* (L.) Merr., and the "wild soybean", *G. soja* Sieb. & Zucc., constitute the subgenus *Soja*. Both species are annuals with a diploid number of 2n=40 (HADLEY & HYMOWITZ, 1973). These two species appear to be genetically compatible, and hybrids between the two can be readily obtained (AHMAD et al., 1977; NEWELL, 1986; WILLIAMS, 1948). The subgenus *Glycine*, which is currently undergoing taxonomic revision, is provisionally comprised of twelve species (HYMOWITZ, 1987; NEWELL, 1986).

All members of the subgenus *Glycine* are perennial, twining or scrambling herbs with a thick tap root. These plants grow in such diverse environments as arid zones, alpine regions and tropical areas (BROUÉ et al., 1982; BURDON & MARSHALL, 1981b). Physiological traits possessed by the wild species include drought, heat and cold tolerance, daylength neutrality and salt tolerance (BROUÉ et al., 1982; HYMOWITZ, 1986; NEWELL & HYMOWITZ, 1982). Screening of the perennials has shown them to carry resistance for leaf rust, *Phakopsora pachyrhizi* Syd., yellows mosaic virus, *Phaseolus virus* 2 and powdery mildew, *Erysiphe polygoni* D.C. (BURDON & MARSHALL, 1981a; BURDON & MARSHALL, 1981b; NEWELL AND HYMOWITZ, 1982; SHANMUGASUNDA-

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version of GAMBORG et al.'s (1968) B-5 medium, they recovered seven, viable hybrid plants. Six of these were from *G. max* cv. Altona x *G. tomentella* IL 428 (University of Illinois No.) (2n=78) and one from *G. max* cv. Altona x *G. tomentella* P.I. 441.002 (2n=80). The medium used included the following additions per liter: 0.17 g potassium dihydrogen phosphate, 1.65 g ammonium nitrate, 2 mg glycine, 1 mg 6-benzylaminopurine and 0.64 g 6-furfurylamino purine. Sucrose was included in the medium at a concentration of 100g/l. The same medium and culture techniques were used by SINGH & HYMOWITZ (1985) to obtain a hybrid between *G. tomentella* P.I. 483.224 x *G. max* cv. Clark.

Also in 1982, BROUÉ et al. reported the recovery of five interspecific hybrids resulting from a cross between a synthetic amphiploid of ((*G. tomentella* (1316) x *G. canescens* (1232)) x *G. max* cv. Lincoln. Both ovules and embryos were cultured using the "nurse endosperm" technique developed by DELATOUR et al. (1978). The medium used was that of WILLIAM'S (1978), which has a low nutrient salts concentration, a high level of vitamins, sucrose at 15 g/l and no hormones.

GRANT et al. (1984) successfully cultured perennial hybrid embryos and ovules on several media types, including that used by BROUÉ et al. (1982). An artificial medium developed by BOURGIN & NITSCH (1967) was also used, as was a version of MURASHIGIE & SKOOG'S (1962) medium, modified

by the addition of minor salts at ten times the reported rate, 0.018 mg/l 1-naphthalene acetic acid and 2.19 mg/l zeatin. Sucrose was included in all media types at the rate of 30 g/l.

Interspecific hybrid soybean embryos have been successfully cultured using various media, culture techniques and regimes, with rescue success ranging from 1 to 4% (NEWELL, 1986). RAGAHAVEN & TORREY (1963) and NORSTAG (1979) have suggested that sucrose level and concentration and plant hormone interactions in embryo culture media are interrelated and highly variable according to plant species and developmental stage of the embryo being cultured. An experiment was conducted to identify an improved nutrient medium for the culture of interspecific hybrids of *G. max* and perennial *Glycine* species. Hybrid embryos were not used for this experiment due to the constraints imposed by the time required to perform the many hand pollinations which would have been necessary to insure that a sufficient number of hybrid embryos would be available for culturing. Crossing success rates for interspecific hybridizations between soybean and perennial species is less than 10%. Further, the interval of time between pollination and pod abortion is variable among hybrids and would have confounded the situation of having an unreliable supply of suitable material.

The objectives of this research were to identify the

factors in culture medium which have the greatest effect on survival of immature embryos of *G. max* and two of the perennial *Glycine* species, *G. tabacina* and *G. tomentella*.

MATERIALS AND METHODS

The three genotypes used in the study were *G. max* cv. Bay, *G. tabacina* P.I. 339.661 (2n=40) and *G. tomentella* P.I. 339.661 (2n=78). Bay and the *G. tomentella* accession were chosen since we had obtained hybrids of these genotypes in an earlier study. The *G. tabacina* accession was selected at random from among the perennials available to us at the time. Seeds of the perennials were obtained from the USDA Germplasm Bank in Urbana, Illinois. The source plants used for this study were grown in pots in the greenhouse under 16-hour days, supplemented, when necessary, with illumination from Sylvania 1000 watt, high-pressure sodium, Maxi-Grow Batwing lamps. Greenhouse temperatures were maintained around 21° C night and 27° C day.

Beginning in the summer of 1985, 20 pots of Bay soybeans were planted every 12 to 14 days. Four seeds were sown into 2-liter pots containing a 50:50, soil:peat mixture, which was inoculated with rhizobium bacteria at the time of planting. Seedlings were thinned to one per pot. Perennial species, which had been maintained since planting in 1983, were grown in 4-liter pots in the same potting medium mentioned above, except that the soil mixture was not inoculated. The perennials were fertilized every 3 to 4 months with 'Osmacote' 14-14-14:N-P-K, a time-released fertilizer.

The experiment, regarded as fixed, was designed as a

complete factorial using the nutrient salts composition of both MURASHIGIE & SKOOG'S (1962) (MS) medium and GAMBORG et al.'s (1968) B-5 (B5) medium in combination with the vitamin components of MS, B5 and WILLIAMS' (1978) (WW) medium. The major nutrient salts of both MS and B5 media were tested at standard, one half the standard and two times the standard concentration along with sucrose concentrations of 15, 30, 60 and 120 g/l. A total of 72 media types were tested, with twelve combinations of major salts:sucrose concentrations for each of the six nutrient salts:vitamin combinations.

The experiment was divided into three parts due to the time constraints imposed by media preparation, embryo-plating and note-taking. Twenty-four media combinations were tested in each set of the experiment. The first set consisted of media types having MS salts as a base, with twelve media using MS vitamins and the other twelve, WW vitamins. Media with B5 salts, twelve in combination with B5 vitamins and twelve with WW vitamins, comprised the second set of media tested. The third set of media included twelve media with MS salts and B5 vitamins and twelve with B5 salts and MS vitamins.

A 60 x 115 mm trident petri dish, containing one of the 72 media types, was considered to be the experimental unit. Two embryos of each genotype were cultured in one section of the dish. Each medium combination was repli-

cated four times in the first two sets and three times in the third set. Therefore, eight embryos of each genotype were tested on each of the 48 media of the first two sets combined, while six embryos per genotype were cultured on each of the 24 media tested in the third set. The total number of embryos per genotype cultured on media containing MS salts and either MS or WW vitamins was 96, as it was for media containing B5 salts in combination with either B5 or WW vitamins. A total of 72 embryos of each of the three genotypes were tested on media containing either MS salts and B5 vitamins or that containing B5 salts and MS vitamins.

Pods were removed from plants at a developmental stage comparable to that of hybrid pods at the time of embryo rescue. Pods of Bay ranged in size from 1 to 2 cm, 0.8 to 1.5 cm for *G. tomentella* and 0.5 to 1.5 cm for *G. tabacina*. Prior to embryo excision, pods were surface-sterilized by immersion in 70% ethanol followed by a one-minute soak in a solution of 20% 'Clorox' bleach (5.25% sodium hypochlorite):0.05% 'Sigma' Nonidet P-40. They were allowed to air-dry before dissection under sterile conditions. An attempt was made to place embryos of similar developmental stages for each of the genotypes in the same petri dish. At time of plating, embryos were measured from radical initials to cotyledonary initials, and ranged in length from 0.2 to 1.5 mm across genotypes.

Dishes were sealed with 'Parafilm' to reduce evaporative moisture loss from the medium and to discourage contamination of the cultures. The dishes were kept in a 'Percival' model I-35-LVL controlled environment chamber maintained at a constant temperature of 22° C and a 12-hour day at 60 $\mu molm^{-2}s^{-1}$. Developing embryos were transferred to fresh medium of the same type approximately every 4 weeks for a total of 3 transfers.

The length of germinating embryos from apex to radical tip was recorded at the time of each transfer. Expansion of primary and trifoliate leaves, as well as radical length and secondary root formation, was also recorded as plants developed. Embryos which failed to increase in size within two months were considered to be dead.

One month after the third transfer, cultured embryos were scored as plants,and given a score of one, or as dead, and given a score of 0. Embryos which germinated and produced at least one trifoliate leaf and a branching root system were considered to be plants. This criterion was based upon the ability of plantlets to survive the transfer from culture to potting in a growing medium. Cultured embryos which failed to reach this stage of development were regarded as dead, even if they were not. Therefore, all data are reported as mean percentages of plant recovery.

Since the experiment was divided into three parts, a

combined statistical analysis was not appropriate. Therefore, each of the first two sets of media tested were analyzed separately using analysis of variance. Because the media tested in the third set did not share a nutrient salts composition, individual analyses were run for each salt:vitamin combination of this set. The general linear model was used for these analyses to account for missing data due to contamination of material, although these losses were minimal. Fisher's Least Significant Difference among means was computed for each of the analyses at the 5% probability level.

While statistically significant differences among all media tested could not be determined, a summarization of results across the entire experiment seemed appropriate. Certain trends appeared when the experiment was viewed as a whole which were not readily apparent when each analysis was considered individually.

A combined correlation coefficient for the size of embryos at the time of plating vs. percent plant recovery was determined across genotypes as well as for each genotype. Differences among mean length at time of plating were computed for all genotypes at the 0.05 and 0.01 level of confidence.

RESULTS AND DISCUSSION

The significant differences in plant recovery rate according to genotype may be accounted for, in large part, by the substantially and consistently lower recovery rates of *G. tomentella* (Table 1). While the greatest number of plants were obtained from cultured embryos of *G. tabacina*, recovery rates for this genotype were significantly higher than for Bay only when media contained MS vitamins. The overall percentage of cultured embryos that were recovered as plants was 31.8% for Bay and 35.0% for *G. tabacina*; however, plants were obtained from only 7.7% of the cultured embryos of *G. tomentella*.

While plant recovery rates were not significantly affected by the vitamin type included in the media, recovery percentages shown in Table 1 indicate that more plants of both Bay and *G. tomentella* were recovered from media containing WW vitamins. *G. tabacina* embryos developed into plants more frequently when media contained MS vitamins regardless of the nutrient salt formulation.

The concentration of sucrose in embryo culture media had a significant effect on plant recovery rates for all salt:vitamin combinations. These differences in mean recovery rates according to sucrose concentration (Table 2) were likely influenced by the low plant recovery percentages from media containing 120 g/l sucrose. No plants were recovered from media with sucrose at this

concentration in combination with MS salts, regardless of vitamin type.

Overall, the greatest number of plants were obtained from embryos cultured on media containing sucrose at 30 g/l. Media having a sucrose concentration of 15 g/l showed the second highest recovery rates, followed by those with 60 g/l sucrose. Recovery percentages at these sucrose concentrations, in the order mentioned above, were 35.8, 28.7 and 26.9. Only 7.4% of embryos cultured on media containing 120 g/l sucrose developed into plants. The higher plant recovery rate of 49.9% from B5 salts:WW vitamin media types with 60 g/l vs. 36.1% from those with 15 g/l sucrose was the only significant contradiction of this trend.

The reaction of the individual genotypes to the sucrose concentration of the media was similar to that of cultured embryos across genotypes. With few exceptions, a greater number of plants of both of the perennial accessions were obtained from media with 30 g/l sucrose, while Bay performed better on media containing sucrose at the 15 g/l level (Table 2).

Even though statistical comparisons cannot be made for the differences in plant recovery in relation to the salt composition of the medium, the overall mean recovery percentages on Table 2 demonstrate that more plants were obtained from media containing B5 salts than from those

containing MS salts. This was true for each genotype as well as for overall recovery rates.

The differences in concentration of major nutrient salts significantly affected mean plant recovery rates, which were greatest when embryos were cultured on media with the standard (1X) concentration of major salts (Table 3). The higher percent plant recovery from media types with a B5:WW, salts:vitamin composition and a 2X concentration of salts was not significantly better than from these types having a 1X concentration. The summarized data in Table 3, confirmed that the average percentage of plants recovered was greatest at a 1X salt concentration. This was also true for each genotype. The plant recovery rates for Bay and *G. tabacina* were both 41.9% at this concentration, which allowed a 10.1% recovery of *G. tomentella* embryos.

Tables 4 and 5 show plant recovery for each genotype according to individual media, and indicate that the three highest overall recovery percentages were obtained from media containing B5 salts (Table 4). The greatest percentage of plants obtained from any medium tested was 79.2% of cultured embryos recovered from medium containing B5 salts, WW vitamins, 30 g/l sucrose and the standard (1X) concentration of major nutrient salts (Table 4). The greatest percentage of *G. tomentella* plants recovered from any medium were from this formulation, while embryos of Bay

and *G. tabacina* showed their second highest recovery rates of 87.5% from this medium.

The greatest number of plants obtained from any medium containing MS salts, 58.3%, contained the same vitamin composition and sucrose and salts concentrations of the top-ranking medium with B5 salts. Second- and third-ranking media types both contained B5 salts, with sucrose:salts concentrations of 15 g/l:0.5X, although the second-ranked medium contained WW vitamins while the third-ranked contained B5 vitamins.

GAMBORG et al.'s B-5 medium, with B5 salts and vitamins, 30 g/l sucrose and 1.0X salt concentration, showed a recovery rate of 58.3% (Table 4), the fourth highest rate for all media tested. However, the plant recovery for *G. tomentella* was only 12.5%, while 100% of the *G. tabacina* embryos plated on this medium developed into plants. MURASHIGIE & SKOOG'S medium (MS salts and vitamins:30 g/l sucrose:1.0X salts) ranked fifth overall, with a mean recovery percentage of 54.2% (Table 5).

The standard MS and B-5 media, along with five others (Tables 4 & 5), were selected for further testing using interspecific hybrids embryos and their parentals. Criterion for selection of these media was based upon recovery rates of individual genotypes rather than mean recovery rate. An exception to this was the choice of the medium containing MS salts, WW vitamins and a sucrose:salts

concentration of 60 g/l:1.0X. This was the original medium we had used successfully for the culture of interspecific hybrid embryos.

There was significant correlation at the 1% level between embryo length at time of plating and recovery percentage. The individual coefficients of 0.53 for Bay, 0.59 for *G. tabacina* and .37 for *G. tomentella*, and the overall correlation coefficient of 0.51 indicated that some of the variability in plant recovery rate was due to the initial size of the plated embryo. Mean length of embryos at time of plating for each genotype was 0.87 mm for Bay, 0.77 mm for *G. tabacina*, and 0.74 mm for *G. tomentella*. Tests for significant differences among these means at the 5% level of confidence revealed that embryos of Bay were significantly larger than those of either perennial. There were no differences in embryo length between the perennials nor among any of the genotypes at the 1% level.

Results indicate that a sucrose concentration of 120 g/l, regardless of the type or concentration of other medium components, is too great for efficient plant recovery from cultured embryos of the three genotypes tested. Further, the genotypic differences in recovery rates, according to media composition, indicate that it may be necessary to perform preliminary experiments to identify the levels of medium components most suitable for the *Glycine* species being cultured. However, recovery rates of

more than 50% for each of the genotypes tested were achieved on one of the media tested. Therefore, it seems possible to identify a medium from which cultured embryos of several *Glycine* species and their hybrids could be recovered as plants at an acceptable level for all, though optimum for none.

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TABLES

Table 1. Plant recovery of cultured embryos of three Glycine genotypes as related to the salt and vitamin composition of the culture media.

Genotype	Percentage of cultured embryos recovered as plants						
	B5 salts			MS salts			Mean
	Vitamin			Vitamin			
	B5	MS	WW	B5	MS	WW	
G. max cv. Bay	52.1	30.5	55.9	6.9	15.4	29.9	31.8
G. tabacina 339.661	56.2	44.4	46.9	8.3	30.8	23.6	35.0
G. tomentella 339.657	8.3	5.5	15.9	0.0	7.7	8.7	7.7
Mean	38.9	26.8	39.6	5.1	18.0	20.7	
$LSD_{.05}$	10.8	12.2	10.8	6.7	9.2	9.2	

Table 2. Plant recovery of cultured embryos of three Glycine genotypes as related to the sucrose concentration of media having different compositions of salts and vitamins.

Sucrose Concentration (g/l)	Genotype	Percentage of cultured embryos recovered as plants						
		B5 salts			MS salts			Mean
		Vitamin			Vitamin			
		B5	MS	WW	B5	MS	WW	
15								
	G. max cv. Bay	75.0	50.0	54.1	22.1	20.0	41.6	43.8
	G. tabacina 339.661	41.6	44.4	33.3	11.1	32.4	37.5	33.3
	G. tomentella 339.657	23.4	5.5	20.8	0.0	5.1	0.0	9.1
	Mean	46.7	33.3	36.1	11.1	19.2	26.4	28.7
30								
	G. max cv. Bay	62.5	44.4	65.4	0.0	25.0	50.0	41.2
	G. tabacina 339.661	75.0	61.1	66.7	16.7	54.1	29.1	50.5
	G. tomentella 339.657	4.1	16.7	29.1	0.0	25.0	20.0	15.8
	Mean	47.2	40.7	53.7	5.6	34.7	33.0	35.8
60								
	G. max cv. Bay	45.8	27.7	70.8	5.6	16.7	27.9	32.4
	G. tabacina 339.661	70.8	55.5	70.8	5.6	33.3	27.8	44.0
	G. tomentella 339.657	4.1	0.0	8.3	0.0	0.0	13.9	4.4
	Mean	40.2	27.7	49.9	3.7	16.7	23.2	26.9
120								
	G. max cv. Bay	25.0	0.0	33.3	0.0	0.0	0.0	9.7
	G. tabacina 339.661	37.5	16.7	16.6	0.0	0.0	0.0	11.8
	G. tomentella 339.657	0.0	0.0	4.1	0.0	0.0	0.0	0.7
	Mean	20.8	5.6	18.0	0.0	0.0	0.0	7.4
Overall mean		38.7	26.8	39.4	5.1	17.7	20.7	
$LSD_{.05}$ (Sucrose*genotype) conc.		21.6	N.S.*	21.6	N.S.	18.5	18.5	
$LSD_{.05}$ (Sucrose conc.)		10.8	12.2	10.8	6.7	9.2	9.2	

* Not significant at the 5.0% level.

Table 3. Plant recovery of cultured embryos of three Glycine genotypes as related to the major nutrient salt concentration of media having different compositions of salts and vitamins.

Major salts concentration	Genotype	Percentage of cultured embryos recovered as plants						
		B5 salts			MS salts			Mean
		Vitamin			Vitamin			
		B5	MS	WW	B5	MS	WW	
0.5X								
	G. max cv. Bay	40.6	20.8	39.7	4.2	20.5	18.7	24.1
	G. tabacina 339.661	56.2	20.8	53.1	12.5	40.1	34.3	36.2
	G. tomentella 339.657	9.3	4.1	15.6	0.0	10.0	9.3	8.1
	Mean	35.4	15.2	36.1	5.6	23.5	20.8	22.8
1.0X								
	G. max cv. Bay	59.3	37.5	68.7	8.3	22.3	55.2	41.9
	G. tabacina 339.661	75.0	45.8	59.3	12.4	40.1	18.7	41.9
	G. tomentella 339.657	3.1	12.5	18.7	0.0	9.3	16.7	10.1
	Mean	45.8	31.9	48.9	6.9	23.9	30.2	31.3
2.0X								
	G. max cv. Bay	56.2	33.3	59.3	8.3	3.1	15.6	29.3
	G. tabacina 339.661	37.5	59.3	28.1	0.0	9.3	17.7	25.3
	G. tomentella 339.657	11.3	0.0	12.5	0.0	0.0	0.0	4.0
	Mean	35.0	30.9	33.3	2.8	4.1	11.1	19.5
Overall mean		38.7	26.8	39.4	5.1	17.2	20.7	
$LSD_{.05}$ (Salt*genotype) conc.		18.8	21.2	18.8	N.S.*	18.5	18.5	
$LSD_{.05}$ (Salt conc.)		10.8	12.2	10.8	N.S.	9.2	9.2	

* Not significant at the 5.0% level.

Table 4. Plant recovery of embryos of three Glycine genotypes cultured on media containing B-5 nutrient salts as related to vitamin composition and the concentration of sucrose and major salts.

Vitamin type	Sucrose conc. (g/l)	Major salts conc.	Percentage of cultured embryos recovered as plants			
			Genotype			Mean
			G. max cv. Bay	G. tabacina 339.661	G. tomentella 339.657	
B5	15	0.5X	87.5	62.5	37.5	62.5*
		1.0X	75.0	37.5	0.0	37.5
		2.0X	62.5	25.0	37.2	40.1
	30	0.5X	50.0	37.5	0.0	29.2
		1.0X	62.5	100.0	12.5	58.3*
		2.0X	75.0	87.5	0.0	54.2
	60	0.5X	25.0	75.0	0.0	33.3
		1.0X	62.5	100.0	0.0	54.2
		2.0X	50.0	37.5	12.5	33.3
	120	0.5X	0.0	55.7	0.0	18.6
		1.0X	37.5	56.7	0.0	31.4
		2.0X	37.5	0.0	0.0	12.5
	Mean		52.1	56.2	8.3	38.7
MS	15	0.5X	50.0	33.3	0.0	27.8
		1.0X	50.0	50.0	16.7	38.9
		2.0X	50.0	50.0	0.0	33.3
	30	0.5X	33.3	50.0	16.7	33.3
		1.0X	33.3	66.7	33.3	44.4
		2.0X	66.7	66.7	0.0	44.4
	60	0.5X	0.0	0.0	0.0	0.0
		1.0X	66.7	66.7	0.0	44.4
		2.0X	16.7	100.0	0.0	38.9
	120	0.5X	0.0	0.0	0.0	0.0
		1.0X	0.0	0.0	0.0	0.0
		2.0X	0.0	50.0	0.0	16.7
	Mean		30.5	44.4	5.5	26.8
WW	15	0.5X	75.0	87.5	43.1	68.5*
		1.0X	50.0	12.5	1.7	21.4
		2.0X	37.5	0.0	12.5	16.7
	30	0.5X	58.8	50.0	12.5	40.4
		1.0X	87.5	87.5	62.5	79.2*
		2.0X	50.0	62.5	12.5	41.7
	60	0.5X	25.0	75.0	0.0	33.3
		1.0X	87.5	100.0	0.0	62.5
		2.0X	100.0	37.5	25.0	54.2*
	120	0.5X	0.0	0.0	0.0	0.0
		1.0X	50.0	37.5	12.5	33.3
		2.0X	50.0	12.5	0.0	20.8
	Mean		55.9	46.9	15.9	39.4

* Selected for futher testing using hybrid embryos.

Table 5. Plant recovery of embryos of three Glycine genotypes cultured on media containing MS nutrient salts as related to vitamin composition and the concentration of sucrose and major salts.

Vitamin type	Sucrose conc. (g/l)	Major salts conc.	Percentage of cultured embryos recovered as plants			
			Genotype			Mean
			G. max cv. Bay	G. tabacina 339.661	G. tomentella 339.657	
B5	15	0.5X	16.5	0.0	0.0	5.6
		1.0X	33.3	33.3	0.0	22.2
		2.0X	16.7	0.0	0.0	5.6
	30	0.5X	0.0	50.0	0.0	16.7
		1.0X	0.0	0.0	0.0	0.0
		2.0X	0.0	0.0	0.0	0.0
	60	0.5X	0.0	0.0	0.0	0.0
		1.0X	0.0	16.7	0.0	5.6
		2.0X	16.7	0.0	0.0	5.6
	120	0.5X	0.0	0.0	0.0	0.0
		1.0X	0.0	0.0	0.0	0.0
		2.0X	0.0	0.0	0.0	0.0
	Mean		6.9	8.3	0.0	5.1
MS	15	0.5X	58.2	47.9	15.1	40.5
		1.0X	1.7	35.0	1.7	12.8
		2.0X	0.0	14.3	0.0	4.3
	30	0.5X	25.0	62.5	25.0	37.5
		1.0X	37.5	75.0	50.0	54.2*
		2.0X	12.5	25.0	0.0	12.5
	60	0.5X	0.0	50.0	0.0	16.7
		1.0X	50.0	60.0	0.0	33.3
		2.0X	0.0	0.0	0.0	0.0
	120	0.5X	0.0	0.0	0.0	0.0
		1.0X	0.0	0.0	0.0	0.0
		2.0X	0.0	0.0	0.0	0.0
	Mean		15.4	30.8	7.7	17.2
WW	15	0.5X	37.5	62.5	0.0	33.4
		1.0X	87.5	25.0	0.0	37.5
		2.0X	0.0	25.0	0.0	8.3
	30	0.5X	37.5	50.0	12.5	33.3
		1.0X	100.0	25.0	50.0	58.3
		2.0X	12.5	12.5	0.0	8.3
	60	0.5X	0.0	25.0	25.0	16.7
		1.0X	33.5	25.0	16.9	25.1*
		2.0X	50.2	33.5	0.0	27.9
	120	0.5X	0.0	0.0	0.0	0.0
		1.0X	0.0	0.0	0.0	0.0
		2.0X	0.0	0.0	0.0	0.0
	Mean		29.9	23.6	8.7	20.7

* Selected for futher testing using hybrid embryos.

ABSTRACT

FACTORS AFFECTING PLANT RECOVERY OF CULTURED EMBRYOS OF THREE *GLYCINE* GENOTYPES

by

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ABSTRACT

Embryos of *Glycine max* (L.) Merr. cv. Bay, *G. tabacina* (Labill.) Benth. and *G. tomentella* Hayata were cultured on 72 different media in a complete factorial experiment using the nutrient salts of both MURASHIGIE & SKOOG (1962) medium and GAMBORG et al.'s (1968) B-5 medium; vitamin components of MS, B5 and WILLIAMS' (1978) medium; major nutrient salts at three concentrations and sucrose at four concentrations to determine media components which affected plant recovery rates and to identify a medium which would allow a greater percentage of plant recovery from cultured embryos of *G. max* x various perennial *Glycine* species than previously reported.

Genotypic differences for plant recovery were significant in all analyses of the experiment, with *G. tomentella* having substantially lower recovery rates than either of the other two genotypes, whose recovery rates were similar. Mean plant recovery percentages were greatest when the medium contained B5 salts and WW vitamins. Very few plants of any genotype were obtained from media containing 120 g/l sucrose, regardless of the type or level of other media components, indicating that this concentration is too great for efficient plant recovery of the genotypes tested. The standard concentration of major nutrients salts allowed higher recovery rates for all genotypes.

The greatest mean plant recovery rate of any media

tested was 79.2%, as opposed to 25.1% plant recovery from the medium we had previously used successfully. Recovery by genotype from this medium was 87.5% for both Bay and *G. tabacina* and 62.5% for *G. tomentella* as compared with 33.5% of Bay, 25.0% of *G. tabacina* and 16.9% of *G. tomentella* cultured embryos being recovered as plants from our former medium.

Index words: *Glycine max*, *Glycine tabacina*, *Glycine tomentella*, soybean, interspecific hybrid, wide hybridization, embryo culture, media