Evaluation and Selection of Elite Clonal Genotypes of the Sweet Crop Licorice (Glycyrrhiza glabra) in a New Environment

NAGAT A. MOUSA¹, P. SIAGURU¹, S. WIRYOWIDAGDO² and M.E. WAGIH¹,3*

¹Biotechnology Centre, University of Technology, Lae, PNG, North of Australia
²Department of Pharmacology, University of Hasanuddin, Makassar, Indonesia
³Department of Genetics, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

ABSTRACT

The adaptability and vigour of exotic licorice, Glycyrrhiza glabra, as a source of sugar substitute in the subtropics of Papua New Guinea (PNG) was monitored from seed germination to 3-month in the greenhouse and to 12-month in the field. The germination potential of sulphuric acid-scarified seeds was significantly (p>0.01) higher than un-scarified. A total of 120 early-germinated seeds were selected and maintained in greenhouse for 3-month. In a stepwise manner, 65 plants with Biomass Fresh Weight (BFW) = average were initially selected, out of which 36 with Root Fresh Weight (RFW) = average were further selected, from which 12 plants with Root Bulk Density (RBD) = average + SD were finally selected; namely P2, P5, P14, P23, P33, P37, P49, P58, P83, P94, P112 and P118. Clonal stock lines of the 12 selected genotypes (L2 to L118) were established by shoot culture. The agronomical vigour of the clonal genotypes was tested at four time points over 12-month under open field conditions and rainfall averaged 275 mm/month and frequent dry spills. Means of parameters measured increased over the growth period, where rootstock grew steadily, and then growth was directed to shoot recovery until reached a balance. Based on a novel value named Principle Selection Index, (PSI=RFW x RBD), three genotypes including L58 followed by L112 and subsequently L33 were selected and multiplied to a clonal stock in vitro. No common direct relation was found between the vigour and physiological attributes to drought tolerance. However, the low stomata area of L33 and L58 may have played a role in their vigour, especially L58, which showed extremely low peroxidase activity, a characteristic of higher level of drought tolerance, compared to that of L33 and L112. The three genotypes were the lowest in chlorophyll “a” and “b” content showing no special evidence of better photosynthetic apparatus compared to the other genotypes. The agro-physiological advantages of the selected lines, especially L58 suggest their potential as a new crop, at least in the low land of PNG for its economically important rootstock as a source of sugar-substitute in dietary food.

Key words: Dietary food, Glycyrrhiza glabra, licorice, Principle Selection Index, sugar substitute

INTRODUCTION

Licorice, Glycyrrhiza glabra of the family Leguminosease, is a perennial cross-pollinated plant of grassy or semi-bushy type (Olukoga and Donaldson, 1998). The economically important part of the plant is the rootstock, as it contains the Glycyrrhizin; an important substance used as a non-nutritive sweetener, 50-93 times sweeter than sugar; besides a number of important anti-allergic and anti-inflammatory compounds (Duke, 1981; Olukoga and Donaldson, 1998; Subramoniam and Pushpangadan, 1999).

The crop has been cultivated for many years in parts of Europe, China, Mongolia and the Soviet Union and the Mediterranean areas (Duke, 1981). While the crop is not among the flora of Papua New Guinea (PNG), its successful cultivation and adaptability to a wide range of environments, ranging from temperate to tropical, enhances the viability of its performance in PNG. Dry sandy soil and long and hot summer are ideal for licorice (Kukreja et al., 1997).

Duke (1981) described the botanical characteristics of the plant in details. It is characterized by compound pinnate leaves that appear in groups of 9-17 leaflets on upright stems, with oil glands, which make the leaves sticky, exhibiting an erect inflorescence (flower stalk) and axillary clusters of white, lavender, or blue flower spikes with flat pods. The growing
height reaches 20-60 inches. The roots consist of a main vertical tap root grows to depths of 2.0 - 3.5m and several metres wide, capable of fixing atmospheric nitrogen. The roots are soft, fibrous, bright yellow colour inside, and often with several branches of runners and rhizomes (Olukoga and Donaldson, 1998).

Growers usually rely on vegetative propagation for producing uniform or clonal material and rarely use seeds as to avoid variability and delayed germination due to its hard testa (Gupta et al., 1997). Vegetative cultivation involves cuttings of roots, runners and rhizomes with at least a single bud (Bezzi and Aiello, 1996; Olukoga and Donaldson, 1998; Duke, 1981 and Poehman, 1977). Using seeds in licorice cultivation requires breaking seed dormancy due to its hard testa (Shamsutdinov, 1996; Gaganidze, 1985). This included mechanical scarification through use of abrasive materials, rushing of seed coat by special rolling presses, sulphuric acid \( (\text{H}_2\text{SO}_4) \) treatment, hot water, etc. Out of this, the most commonly executed method is the \( \text{H}_2\text{SO}_4 \) treatment. As reported by Gupta et al. (1997), pre-treating the dormant seeds with concentrated \( \text{H}_2\text{SO}_4 \) for five minutes gave best germination result. But prior to this, Verma (1996) followed the similar procedure, however, increased timing to twenty minutes and had a surprising 100% germination.

Despite in field trials reports on root yield varied among workers from 9.8 to 20 tones per hectare (Marzi, 1996; Bezzi and Aiello, 1996; Leto et al., 1996; Milia et al., 1996; Mastro et al., 1993; Marzi et al., 1993), a direct yield comparison may not be valid due to the various different growth factors among those trials. However, in a comprehensive pot trial, Mastro et al. (1993) reported a mean root weight of 81.4 g in the first year but this increased markedly to 753, 1534 and 1930 g, respectively, in the following three years.

This work aimed at studying some agronomical and physiological attributes to the performance of licorice and selection of superior genotypes under the new environment of PNG.

**MATERIALS AND METHODS**

**Germination of seeds and selection of early germination**

Seeds of an exotic licorice, *Glycyrrhiza glabra*, obtained from Richters Herbs, Goodwood, Ontario, Loc 1A0, Canada, were sown in germination trays as soon as they were received and kept at 25°C in the dark. Germination rate was monitored over a period of 28 days for 200 scarified and 200 non-scarified seeds. Plantlets of 120 early germinating seeds from both treatments were selected and advanced.

Scarification to overcome dormancy due to hard seed testa was carried out with 100% \( \text{H}_2\text{SO}_4 \) for 5 min followed by rinsing in distilled sterile water for 15 min prior to planting as described by Gupta et al. (1997). In an open greenhouse, seeds were planted in small pots with sterilized topsoil, farmyard manure and rock sand at a ratio of 1:1:1 and at a depth of about 1 - 2cm.

**Evaluation of 3-month-old plants under greenhouse conditions**

The 120 greenhouse-grown plants were evaluated, and of which 12 plants (10%) were selected and cloned *in vitro* by shoot culture. The selection was carried out on 3-month-old plants based on main agronomical growth parameters of whole plant, root and shoot. Whole plant growth *parameters include* biomass fresh weight, BFW (g). *Shoot growth parameters include* shoot fresh weight, SFW (g) and plant height, PH (cm). *Root growth parameters include* Root Fresh weight, RFW (g), Root dry weight RDW (g), Root volume, RV (ml) and Root bulk density, RBD (g ml\(^{-1}\)), calculated as the ratio of root dry weight over root volume (RFW/RV). Also, observations were made on the tolerance of plants to drought spills, naturally occurring disease infection, and pest infestation.

**Procedure for *in vitro* shoot culture**

Shoot culture was initiated from single-bud-cuttings of the 3-month-old greenhouse-grown plants regenerated from seeds. Explants were surface sterilized with 2.0 % sodium hypochlorite (commercial bleach) for 15 min and washed thrice with sterile distilled water prior to culturing on MS basal medium (Murashige and Skoog 1962). At this stage, no growth regulators were used to optimize growth conditions. After four weeks of growth *in vitro*, shoot and root parameters and percentage of complete plantlets generated were measured. Leaves were then trimmed prior transferring plants to the greenhouse for two weeks of hardening then transferring to the field.

**Field location, soil and weather**

The field was that of the University of Technology Agriculture Farm in Lae, located at an altitude of 54 m above sea level and magnitude 6° 45’ South, 147° East. The farm soil is generally loamy sand (Muneer, 2002), and the farm received an average annual rainfall of 3,294 mm in the year 2000. Short drought occurred from September to February with an average monthly rainfall of 120 - 300 mm/month (Weather Station, University of Technology, Morobe Province).

**Field preparations and experimental details**

Clonal stock of twelve (12) genotypes of licorice regenerated by shoot-culture were evaluated in the new environment using a completely randomized block design (RCBD) with four replicate plots where each of the 12 genotypes was represented by four plants per plot. Prior to planting, the plots were hand-weeded and ploughed. Planting was carried out in mid May 2000 by placing 6-weeks-old plants (spent 4-weeks *in vitro* and 2 weeks hardening in greenhouse)