Technology for Conservation and Species Restoration of Critically Endangered Medicinal Plant of the Desert – *Commiphorawightii*(Gugul) developed by AFRI, Jodhpur

A- A live Commiphorawightii tree in the wild; **B** - Embryogenic callus of Guggul; **C**- Maturing somatic embryos; **D**- Germination of somatic embryos; **E**- *In vitro* hardening of somatic embryo derived plantlets (emblings); **F**- A complete somatic embryo derived plant with healthy tap root system; **G -***Ex vitro* hardening of emblings; **H**- Plantlets under green shade house; **I**- Somatic embryo derived plants successfully transferred to open field.

*Commiphorawightii (*Arn.) Bhandari, commonly known as guggal, is a medicinally important desert species of the family burseraceae. It is a well known for its valuable active principle found in its oleo-gum-resin (guggulsterone E and Z), which are used in drugs preparation for lowering the cholesterol level in human body. *C. wightii* is a red-listed plant species declared under threatened category (IUCN, 2010). Due to over-exploitation, the plant is on the verge of extinction. The technology described here deals with the use of tissue culture technology as a viable alternative for propagation and conservation of this valuable plant.

For the development of tissue culture based protocols, standard tissue culture procedures and laboratory standards were followed. Mother plants were selected from four sites in Rajasthan. These were, Kaylana area and JNV University (both in Jodhpur district), Mangliawas (Ajmer district) and Charbhuja (Rajasmand district). All media were sterilized by autoclaving at 121°C and 20 psi (137,900 pa) pressure for 15 minutes. All the cultures were aseptically

inoculated and manipulated under a sterile laminar flow hood and incubated in tissue culture racks in an aseptic culture room having a temperature of 25±2°C, 16 hour photoperiod and 1600 lux intensity light (via cool florescent lamps and incandescent light bulbs). The strategy for acclimatization (hardening of tissue culture raised plantlets) for all the three protocols have been same.

Protocol for In vitro propagation through Somatic embryogenesis in brief*:*

Initiation of embryogenic cultures. Unripe fruits were used as a source of immature embryos for establishing the embryogenic cultures. After one month interval, callus mass was achieved on B_5 (Gamborg*et al*, 1968) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and subcultured for callus mass proliferation on the same medium for 3-4 weeks. Callus turned embryogenic after sub-culturing callus on hormone free B_5 medium. Initiated embryogeniccalli with different stages of embryos were maintained on alternatively subculturing on modified MS medium supplemented with hormones indole-3-butyric acid (IBA) and 6-benzylaminopurine (BAP) and on hormone-free medium.

Three years old embryogenic callus was maintained on both medium with alternate subculturing. Secondary and tertiary somatic embryos were also obtained. Cyclic embryogenesis was established and stabilized.

Maturation of Somatic Embryos (SEs). Depleted modified MS medium without any PGRs were used for maturation for reducing the water content available in SEs, which results in better desiccation and dehydration of SEs. It was observed that somatic embryos turned whitish and enlarged in size. Further, they were harvested for germination.

Germination of somatic embryos. Matured white somatic embryos were used for germination of SEs on different concentration of gibberellic acid supplemented in modified MS medium. The highest germination percentage (62.25%) of SEs was observed on modified MS medium supplemented with gibberellic acid as well. Plantlet derived from a somatic embryo (embling) were initially whitish in colour which later on converted in greenish colour in presence of light.Acclimatization was done in a two-step manner.A trial of plants derived from this protocol was established in July, 2010

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