Introduction
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Rice is a most important food crop of India, providing 43% of calorie requirements to more than 70% of Indian population. It is the source of livelihood for millions of rural households and also the backbone of the Indian Agriculture. Total rice production of India is 99000 thousand MT (Rice Profile, 2013) and the share of Uttar Pradesh is about 14354 thousand MT of year 2012-13 with productivity of 24.59 Qt/ha and grown on about 5.90 mha (UP Rice, 2013).

As the demand for rice worldwide is rapidly growing, due to great increase in world’s population that consume rice, the yield of rice must be increased through the development of improved farm practices and genetic improvement.

The rate of growth in rice production has slowed down. This will require acceleration in rice production and solving this problem will entail development in rice varieties, which have higher yield, excellent grain quality and resistant to biotic and abiotic stress (Shukla et al., 2012 a,b).

In this chain of events, SwarnaSub1 is a newly released (August 2009) first abiotic submergence-tolerant, highyielding rice variety in India. SwarnaSub1 incorporates the Sub1 gene into the Indian mega-variety Swarna, making it resilient to flooding of upto 10 days while retaining the desirable traits of the original variety. It is one of the rice varieties which became popular within a very short time. Actually this is an improved version of an existing variety Swarna. Scientists only had to test its tolerance of submergence. Even before SwarnaSub1 was released, different research institutions are already multiplying these seeds. Immediately after release, state governments distributed the seeds to other channels for large-scale multiplication and also disseminate the seeds directly to farmers in target areas. Within one year of release, this
variety has reached more than 100,000 farmers in India (IRRI, 2010). Swarna took 25 years after its release to be planted on six million hectares, reaching its current "mega" variety status, but now scientists are hoping that Swarna Sub1 will reach the same status in only five years and it could entirely replace Swarna and spread to other flood-prone areas all over the country (IRRI, 2010).

Owing to the everincreasing demand of rice worldwide, new varieties with higher yields, multiple resistances to pests and diseases, and with tolerance to stresses such as acidity, salinity and/or flooding are greatly needed. In the last five years remarkable and significant advances in plant molecular biology and gene delivery techniques have led to cellular and molecular approaches to crop improvement. These approaches show promise to increase the efficiency of traditional breeding methods and also provide new unconventional approaches to enhance and build rice economy. The introduction of beneficial genes from other organisms/plants such as those encoding disease and insect resistance via genetic transformation into the rice genome arose from developments in this area. These important steps as dramatic genetic improvement were not possible through breeding and selection.

Considerable improvement made through tissue culture techniques are being applied for varietal development of cereal crops including rice in different countries. Among the techniques, anther culture, protoplast fusion, leaf culture, root culture and dehusked grain culture are important in rice tissue culture to exploit somaclonal variation for the creation of novel rice varieties. Somaclonal variation creates novel variability which can be exploited for crop improvement (Islam et al., 2004).

Somatic embryogenesis in rice has been reported from culture of leaf tissue (Wernicke et al., 1981), root tissue (Abe and Futsuhara, 1985). Ruebet al. (1994) developed a reproducible and efficient procedure for regeneration of rice plants from callus through somatic embryogenesis.
During past few decades, biotechnological techniques such as somaclonal variation, *in vitro* selection, production of doubled haploid lines from anther culture and genetic transformation are being employed in rice development for the creation of novel rice varieties. However, the production of embryogenic calli and its subsequent regeneration are the basic prerequisites for the potential use of these techniques. Successful embryogenic calli induction is depended on many factors such as plant genotype, explant type, culture medium, plant growth regulators and culture environment. In order to develop a reproducible and efficient procedure for callus induction of rice, the influence of 2,4-D and other growth hormone concentration with different combinations and culture media were evaluated in the present study.

**Tissue culture in monocots**

Tissue culture of monocots is difficult compared to dicots. It has been observed that rice produces two types of calli viz., embryogenic and non-embryogenic. The former is produced on the surface region, grows faster and gives rise to plants through somatic embryogenesis *(Vasil and Vasil, 1986)*.

**Submergence property in rice**

Submergence tolerance is defined as the ability of rice plant to survive and continue to grow after being completely submerged in water for several days. Rice plants are less tolerant to submergence at early stage. Submergence tolerant is a genetically determined trait with relatively high heritability that is controlled by one or few genes with major effects and minor modifiers.

Several environmental factors such as duration of submergence, water level, temperature, water turbidity, photon flux density and soil condition affect the tolerance of rice plant to complete submergence *(Jackson et al., 2007)*.
Submergence leads to reduction in oxygen supply, because of slow diffusion rate of oxygen in water and its limited supply to plants. Flooding affects morphological and anatomical changes such as leaf senescence, inhibition of growth in dry mass, faster under water leaf elongation, partial injury or even death of entire plant. Chlorosis of tissue and leaf elongation is promoted by accumulation of ethylene within the shoot (Jackson, 1985). Ethylene induces aerenchyma formation in response to flooding but the effect varies in different genotypes. Ethylene is known to induce elongation through involvement of gibberellins.

Pre-submergence carbohydrate reserve plays an important role in supplying energy required for survival of plant during submergence. Reserve carbohydrate is first converted into simple form by action of hydrolytic enzymes. Amylase plays an important role in degradation of starch under submergence (Dunn, 1974). Older seedlings tend to have large carbohydrate reserves and therefore good survival during submergence (Adkins et al., 1990). Culms of submergence tolerant plants contain starch even after being submerged for 7 days, whereas reserves of susceptible rice cultivars were exhausted during the same period (Mallik et al., 1995; Singh et al., 2001).

Submergence tolerant cultivars accumulate starch at a faster rate and retain an appreciable starch content after de-submergence (Smith et al., 1987).

Submergence tolerance is defined as the ability of rice plant to survive and continue growing after being completely submerged in water for several days. Rice plants are less tolerant for submergence at the early growth stages. Submergence of rice by flash flooding is a major constraint to rice production in Asia (Perata and Voesenek, 2007). It adversely affects farmers living on rice-growing areas in south-east. Submergence has dramatic effect on growth and yield of crop plants. Energy deficit, caused by inhibition of respiration, is one of the biggest problems encountered by rice when subjected to submergence because oxygen supply is hampered (Perata et al., 1997).
The effect of water stress on yield of rice is very pronounced during certain period of growth, called the moisture sensitive periods. The most sensitive periods to water deficiency are flowering and head development. In an experiment conducted in the Philippines, it has been shown that moisture stress early in the growth of the rice reduced tillering, thereby reducing yield. When moisture stress was extended into reproductive phase, yield loss was significant.

**Justification of the work**

The purpose of this study was to develop a reproducible and an efficient procedure for callus induction and plant regeneration of embryogenic calli from mature seeds of Swarna Sub 1 for future genetic transformation studies. Callus induction is the first step in rice transformation to obtain embryogenic callus (Rachmawati *et al.*, 2004). In particular, genotype and the composition of culture medium are important factors for successful embryogenic callus induction and regeneration of plants.

**Objectives of the present study**

Considering the above points in view “Standardization of Tissue Culture Protocols for Transformation Studies in Rice (*Oryza sativa* L.) cv. Swarna *Sub1*” has been taken with the following objectives:

- To standardize protocols for plantlet regeneration of Swarna Sub 1 through various modes of tissue culture.
- To acclimatize and transfer the plantlet obtained through *in vitro* culture to field.
- To assess the yield of Swarna Sub 1 plants obtained through *in vitro* culture techniques.
- To assess any variation in submergence properties of the regenerated plants.