Chapter 1

Introduction

1.1 History of groundnut

Classified by a variety of names, including peanut, African nut, Chinese nut, Manila nut, kipper nut, Hawks nut, Jar nut, Earth chestnut, monkey nut, goober pea, ground pea, and ground bean, groundnuts are grown all over the world. The groundnut is an annual legume crop that is auto-allo tetraploid (4x=40). The Greek words *arachis*, which means "a legume," and *hypogaea*, which means "below ground," which refers to the underground production of pods, are the sources of the groundnut's botanical name. Groundnuts are special because, following blooming and fertilization, the pegs (gynophores) lengthen and pierce the earth, allowing the fruit to grow and develop into pods that contain one to five seeds. Since groundnuts are a day-neutral plant, 25 to 30 degrees Celsius is the ideal temperature range for plant growth (Weiss, 2000). It is the most important economic oilseed crop grown in many tropical, sub- tropical, and warm temperate regions of the world between 40^{o}N and 40^{o}S latitudes. Archaeological records indicate that it was grown in Peruvian desert oases between 300 and 2500 BC (Weiss, 2000; Smith, 2002). The most diverse wild varieties of *Arachis* species were found in the Gurarani region of Paraguay, eastern Bolivia, and central Bolivia. The first domestication of cultivated groundnut was probably in the Paraguay and Prarana river valleys in the Chaco region of South America. As far as India is concerned, it may be inferred correctly that the Jesuit Fathers, who accompanied Vasco De Gama soon after his initial arrival in India, are responsible for the introduction of plants into this nation, according to Leiberherr (1928). By the first part of the sixteenth century, groundnuts were most likely brought to India (John *et al.*, 1955).

1.2 Economic importance of groundnut

One of the most significant oilseeds in tropical and subtropical nations is groundnut (*Arachis hypogaea* L.). In the globe, it ranks as the third most significant source of vegetable protein, the fourth most significant edible oil, and the thirteenth

most significant food crop. Because groundnut seeds contain high-quality edible oil, easily digested protein, and carbs, they have a great biological value. Roughly 90% of its output comes from underdeveloped nations, and it is cultivated in almost 100 countries. India leads the world in groundnut output (18.6%) and area (32.1%), trailing only China (42.2%). Gujarat, Andhra Pradesh, Tamil Nadu, Maharashtra, Madhya Pradesh, Rajasthan, and Uttar Pradesh are the seven states in India where 75% of groundnuts are farmed. Agricultural data at a glance (2010) states that 7.17 million tons of groundnuts are produced annually on 6.16 million hectares, with an average productivity of 1163 kg/ha. With a 37.10% output share, Gujarat leads all of India, followed by Andhra Pradesh (21.62%) and Tamil Nadu (13.53%). The Saurashtra area of Gujarat produces more than 80% of Gujarat's groundnuts.

1.3 Importance of groundnut in food and feed

The primary uses of groundnuts are as vegetable oil and seeds for human use. The primary storage tissues are cotyledons, which are also an excellent source of nutritional energy, lipids, and protein. Its seeds have 40–50% fat, 20–50% protein, and 10–20% carbohydrate, making them a rich source of edible oils. Raw groundnut seeds are the best source of thiamine and nicotinic acid and are a great source of vitamins E, K, and B-complex. Additionally, groundnut seeds are a good source of dietary nutrients, including magnesium, phosphorus, and potassium. The oil extracted from the crushed seeds is used as fuel, in cooking, and to light lights. Because it has the proper ratio of unsaturated fatty acids, such as linoleic acid (25–35%), and saturated fatty acids, such as oleic acid (40–50%), groundnut oil is regarded as stable and nutritious. A high linoleic acid content will cause hypocholesterolemia. The cooking quality is enhanced and stability is imparted by the greater oleic/linoleic acid ratio. Tocopherol, an antioxidant found in 0.93 mg/g of groundnut oil, keeps oil from going rancid and improves its shelf life. A popular snack item is groundnuts, which can be salted, dry roasted, roasted in their shell, or fresh cooked. Peanut butter, peanut milk, peanut candies, crackers, cookies, wafers, and chocolates are the most often consumed items. Groundnut protein and oil have been used to make cheese analogues used in cream cheese and cheese spread products (NPC, 1990). The groundnut pressings, seeds, green material, straw are used as feed for animals. Defatted groundnut cake contains 44- 69% of protein; which is used for livestock feed, concentrates and mixture. Groundnut shells

are cheap source of fuel, mulch, bedding material for poultry and it is used for cardboard manufacture. The potential of shells as industrial raw material for the production of white coal, enzymes and alcohol is gaining popularity.

1.4 Vitamin A: An Essential Micronutrient

The term "micronutrients" refers to the necessary vitamins and minerals that organisms require in minute amounts. Enzymes, hormones, and other critical molecules required for healthy growth and development must be produced using these components. Regarding the fact that they are hardly necessary, their lack or deficiency results in serious health issues and raises the chance of death from infections and chronic illnesses. Since the body cannot produce micronutrients on its own, dietary food consumption is a natural strategy to treat micronutrient deficiency (MD). Since MD develops gradually over time and its effects are not apparent until irreversible harm has been done, it is frequently referred to as "Hidden Hunger." Iron, iodine, folate, vitamin A, and zinc deficiencies have been recognized as serious health risks.

The components that make up vitamin A are fat soluble. It is composed of unsubstituted β-ionone rings arranged in an isoprenoid chain. When stored as retinyl esters, retinol, retinal, and retinoic acid are the active forms of vitamin A. The development of the human body's physiological systems and metabolism, including immune system maintenance, healthy vision, genetic regulation, reproduction, embryonic development, skin health, bone metabolism, and many other processes, depend critically on vitamin A. The combination of retina and opsin results in the production of rhodopsin pigment, which is a light-sensitive receptor protein necessary for visual phototransduction and low-light vision. Since the body cannot metabolize too much retinol, free movement retinol attaches itself to retinol-binding protein (RBP). Through esterification via lecithin-retinol acyltransferase, bound retinol can be stored in the liver as retinyl-esters. The primary hormonal metabolite/morphogen needed for organogenesis, epithelial cell differentiation, and embryonic development is retinoic acid.

1.5 Vitamin A Deficiency: Causes and Effects

A diet high in carotenoids is associated with a lower incidence of vitamin A deficiency-related illnesses (VAD). VAD is a condition that primarily affects pregnant

women and children aged six to sixty months and is caused by inadequate consumption of a low-vitamin A diet. One of the most serious nutritional health issues in the world, VAD causes xerophthalmia, an ocular manifestation. It encompasses a variety of ocular conditions, including bitots spot, conjunctival xerosis, keratomalacia, night blindness, and irreversible blindness. The first characteristic stage of VAD is night blindness or bitotspot, a condition in which rod cells (cells sensitive to light) degenerate and sight decreases in low light. Reduced blood levels of serum retinol, or retinol in the blood, are associated with night blindness. When serum retinol content is less than 1.0 µmol/L, the first signs of VAD develop; symptoms worsen below 0.35 µmol/L. Worldwide, around 9.8 million pregnant women and 5.2 million preschool-aged children experience night blindness, whereas 19.1 million expecting mothers and 0.19 billion preschool-aged children have extremely low serum retinol levels. (Haskell, 2012; Islam *et al.*, 2016). VAD is also associated with child mortality from diarrhea and measles. Moreover, it hinders the mobilization of iron, which delays growth and lowers immunity to diseases. The main food sources of carotenoids are seeds, fruits, and vegetables; however, many commonly eaten food crops lack sufficient nutrients to meet the needs of the body. Consequently, VAD is still common in developing countries. For a natural and economical solution, biofortifying fruit crops or staple foods can help reduce VAD. Increased intake of β-carotene results in a decrease in the intestinal bioconversion of β-carotene to vitamin A; on the other hand, well-absorbed vitamin A supplements may be toxic and have been linked to negative health effects (Feskanich *et al.,* 2002; Novotny *et al.,* 2010; Haskell, 2012; Oliveira, 2015).

1.6 Practical Utility of Research Problem

CRISPR technology is the best tool for genome editing, because it is faster easier and precise than any other previous techniques. It can be widely utilized in various crops to improve their economic value and agronomic traits. Vitamin A deficiency is a major health problem for over 400 million people (>100 million are children) suffering from the problem. Improved pro-vitamin A content in widely consumed crops is an attractive cost-effective solution to resolve the problem of supplement and fortified foods that's have limited reach.

Objectives

- To optimize tissue culture protocol of Groundnut for editing gene of interest
- Introduction of gRNA construct into Groundnut ex plant through transformation for herbicide resistance
- Re-generation and detection of *in-vitro* mutations induced by CRISPR system