A SHORT REVIEW ON MICROPROPAGATION OF VARIOUS SPICES IN BANGLADESH

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

I, Naimah Fairuz, hereby certify that the following criteria are fulfilled for the manuscript "A Short Review on Micropropagation of Various Spices in Bangladesh":

1. This material is my own original material of review that has never been published beforehand.

2. All of the sources those are utilized are correctly credited (correct citation) along with a proper and justified reference.

Abstract

Spices have been utilized throughout history for flavoring and preserving meals, as well as medicinal and cosmetic uses. Bangladesh's culinary system has always been spice-dominated, with roughly 27 different varieties of spices being often used. Spices are significant contributors to the national economy of many spice-producing, importing, and exporting countries around the world. However, breeding these spices using traditional methods presents numerous challenges. Micropropagation has proven to be an important approach for overcoming these obstacles. This study examines the *in vitro* regeneration of certain widely available spices in Bangladesh and explores the possible explanations for the variations in outcomes observed in each experiment.

Keywords: Spices; *In vitro* culture; Plant regeneration; Micropropagation; Bangladesh.

Dedicated

To

My Friends and Family

For always believing in me

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List of Acronyms

BAP	6-Benzylaminopurine
HCl	Hydrochloric Acid
IAA	Indole-3 Acetic Acid
IBA	Indole-3 Butyric Acid
Kn	6- furfurylaminopurine
mg/l	Milligram/Litre
MS	Murashige and Skoog (1962) medium
NaOH	Sodium Hydroxide
pH	-log [H+]
NAA	Napthalene acetic Acid
2, 4- D	2,4-Dichlorophenoxyacetic acid
GA3	Gibberelic Acid
ABA	Abscisic acid
AgNO3	Silver Nitrate
BINA	Bangladesh Institute of Nuclear Agriculture
gm	Gram
kg	Kilogram
l	Litre
cm	Centimetre
BARI	Bangladesh Agricultural Research Institute
BAU	Bangladesh Agricultural University
dS/m	Decisiemens Per Metre
BCSIR	Bangladesh Council of Scientific and Industrial Research

2ip	2-Isopentyl Adenine
WPM	Woody Plant Medium
BA	Benzyl Adenine

Chapter 1

Introduction

Chapter 1

Introduction

Spices are a significant collection of agricultural products that are practically required in the culinary arts. It is a plant that enhances the flavor, aroma, aesthetic value, and therapeutic value of food, drink, and other products. They can be primarily described as agricultural products that are used in a variety of ways, such as fresh, ripe, dried, broken, powdered, etc. Spices can be found on the entire plant's tops, in the bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas, and styles. (Parveen et al., 2014)**.** Spices are employed in cuisine all around the world. The value of spices as a food or medication cannot be overstated. Nearly all curries, which are prepared using a variety of spices, are well-liked and delicious.

Many nations, notably in Southeast Asia, have been growing spices and herbs for a very long time, with India being by far the largest producer. Some spices are so priceless that they have even been kept confined inside special boxes. The frequently recounted tale of Marco Polo and the spice route to India, as well as Columbus' accidental discovery of America while looking for a different route to India, serve as examples of the historical significance of the spice trade (Chen & Bahna, 2011).

A complete list of substances used as spices is impossible to present, however **Table 1** lists the most popular ones.

Table 1 *Common Spices*

Common spice names						
Allspice	Ginger	Bay leaf	Star anise	pepper	Turmeric	Mustard seed
Garlic	Sage	Jalapeno pepper	Green cardamom	Mace	Almond	Wasabi
Saffron	Basil	Sesame seed	Myrobalan	Thyme	Mint	Chili
Anise	Curry leaf	Caraway seed	Black cardamom	Celery/ Radhuni seed	Vanilla	Nutmeg
Onion	Nigella seed	Licorice	Cayenne	Shallot	Panch phoron	Chives/ scallion
Cinnamon	Oregano	Paprika	Parsley	Dill	Fennel seed	Fenugreek
Clove	Coriander	Cumin seeds	Peppercorn (black, green, pink, white)	Poppy seed	Large cardamom	

Spices have some therapeutic value. For example, turmeric helps lower blood sugar, garlic helps prevent heart disease and preserve memory, and ginger is well recognized for its digestive properties (Nahar, 2017). Even though there is no treatment for COVID-19, patients' immunity to this pandemic is greatly increased by spices and other nutrients (Rana et al., 2021)

With variations in their individual components and quantities, blends of diverse spices are becoming more and more popular.

1.1 Some common spices explored in Bangladesh

1.1.1. Chilli

The Solanaceae family includes the herbaceous flowering plant *Capsicum annuum*. There are about 20 species in the genus Capsicum. One of the most significant vegetable crops in the world is *C. annuum*, also known as pepper, sweet pepper, or bell pepper. Bell peppers come in a variety of colors, including red, green, yellow, brown, and purple. They are a necessary vegetable for the kitchen as well as a condiment and spice that give food color, flavor, zest, and piquancy various foodstuffs (Akther et al., 2020). Ascorbic acid is a key component of peppers, and it has been linked to a number of health benefits. It is in high demand both when it is green and when it is ripe. Due to the abundant synthesis of different carotenoid pigments during ripening, they have attractive colors (Hossain, 2018).

Chilli contains a lot of minerals, like, molybdenum, manganese, phosphorus, and potassium as well as vitamins A, B-complex, C, and E (Hossain, 2018). *Capsicum annuum* is mostly used commercially. They can be used whole, chopped, finely ground, coarsely ground, with seeds included, or none at all. Pickled fruits, chilli sauce, chilli powder (also known as cayenne powder), crushed red pepper flakes, fermented mash, paprika, and three different types of oleoresins are a few examples of processed foods that primarily contain peppers. Fresh and processed salsas, curry powders, barbecue seasonings, chili powder (a blend of chili powder, oregano, cumin, and garlic powder), and many other dishes are examples of processed foods that contain a significant number of peppers. Additionally; it is used in the poultry, cosmetics, and pharmaceutical industries. Diseases like dropsy, diarrhea, asthma, arthritis, muscle cramps, and toothaches are treated with it as a medicine (Nadeem et al., 2011). Furthermore, it has a number of medicinal properties, including those that are anti-inflammatory, analgesic, carminative, and rubefacient. Additionally, it has strong antioxidant, antimutagenic, anti-tumor, hypoglycemic, antifungal, and antimicrobial activities (Hossain, 2018).

1.1.2. Black pepper

Piper nigrum (family Piperaceae) is a very useful medicinal plant. Sanskrit word pippali, which meaning "berry," is the source of the term "pepper". Because of its distinct flavor; it is most commonly used as a spice in the kitchen. It is one of the oldest spices and is known as the "king of spices" because of its medicinal properties (**Table 2**). Black pepper is used in a variety of applications, including treatment, preservatives, and flavorings, the cosmetics industry, the perfume industry, and as a medicinal ingredient, both alone and in combination with other ingredients.

Table 2 *Pharmacological Activities of Piper nigrum (source: Damanhouri and Ahmad, 2014; Joshi et al., 2018)*

Sl. No.	Pharmacological Activities	Sl. No.	Pharmacological Activities
1	Antihypertensive activity	16	tumor reduction activity
2	Anti-asthmatic activity	17	Anti-obesity
3	Cognitive action and Fertility activity	18	Antidiabetic
4	Antimicrobial activity	19	Antipyretic activity
5	Antioxidant activity	20	Anti-adipogenesis activity
6	Anti-cancer activity	21	Effect on enzyme
7	Anti-inflammatory activity	22	Effects in neurodegeneration and cognitive impairment
8	Hepatoprotective activity	23	Bioavailability
9	Anti-diarrheal activity	24	Decrease food transit time
10	Digestive activity	25	Effect in bile secretion
11	Antidepressant activity	26	Effect in pharmacokinetic of drugs
12	Immunomodulatory activity	27	Synergistic effect with drugs
13	Anticonvulsant activity	28	Inhibition of lungs metastatis
14	Analgesic activity	29	Fertility effect
15	Effect of piperine on metabolism	30	Anthelmintic activity

1.1.3. Garlic

Garlic, also known as *Allium sativum*, is an aromatic bulbous plant and an annual herbaceous spice that belongs to the subfamily Amaryllidaceae includes the family Allioideae. It is thought to have its roots in central Asia, particularly the Mediterranean region. The top producers of this crop today are China, Bangladesh, India, South Korea, Spain, Egypt, Thailand, and Turkey. The most frequently consumed part of the plant is the clove, which is typically divided into numerous fleshy sections (raw or cooked). Cloves with a teardrop shape are encased in papers that resemble dry skin and combine to form the bulb. Garlic is a significant crop of spices that is used in many traditional ayurvedic treatments because it is high in protein, carbohydrates, and phosphorus. Supplementing with garlic is known to improve immune system performance and significantly lower blood pressure in those with high blood pressure (Mubarrat et al., 2018).

Garlic is well-known throughout the world as a crucial spice for various dishes. Additionally, it has been utilized for more than 4,000 years in conventional medicine. As a folk remedy, it has a long history. Both raw garlic and products made from garlic extract have antibacterial and cancer-preventing properties. Garlic extract used in water significantly lowers cholesterol levels. It is also a well-liked treatment for a number of illnesses. Garlic is effective in treating lung diseases, desensitization, typhoid, cholera, and infections of the stomach and intestine, according to Unani and Ayurvedic medicine. In recent years, it has been used to prepare garlic oil and garlic powder to add flavor to curries (Parvin et al., 2007).

1.1.4. Ginger

Ginger (*Zingiber officinale* Roscoe) is a rhizomatous monocotyledon that is a member of the Zingiberaceae family. It is a well-known spice that is made from a tropical herbaceous plant's rhizome. Its use for medical purposes dates back more than 2500 years in China and India, where it was first found in 400 BC. (Sumon et al., 2019). It is one of the earliest spices that have been discovered in Europe; the Greeks and Romans acquired it through Arabian traders. Later, it was brought to Africa, the West Indies, and other hotter regions of the world. Ginger is grown all over the world, with India, Sierra Leone, Nigeria, Japan, Nepal, Jamaica, Southern China, Pakistan, Taiwan, Australia, Mauritius, Malaysia, and Indonesia ranking as the top producers. The highest quality ginger is produced in Jamaica and India, then West Africa. India and China are two of the world's top suppliers of ginger (Mamun, 2006).

It is frequently utilized in the production of several food items, including curry powder, some curried meats, table sauces, confections, and ginger ale. It is widely used as a spice, a medication, and an ingredient in many foods and drinks all around the world. In Asian, Indian, and Arabic herbal traditions, it has a long history of usage as medicine. Ginger has been used to treat cardiac ailments, arthritis, colic, and diarrhea. It has been used to treat headaches, painful menstrual cycles, flu-like symptoms, and the common cold (Yesmin et al., 2016). In western nations, ginger is frequently used in foods including pickles, gingerbread, biscuits, cakes, puddings, and soups. Ginger wine, ginger beer, are all made with it (Mamun, 2006).

1.1.5. Mint

The Lamiaceae family, which includes the *Mentha* sps., thrives in humid places of temperate climates. The aromatic and therapeutic herb mint (*Mentha* sp.) is grown extensively for its essential oils all over the world. Mint is a good source of vitamins A and C, as well as a minor bit of B2. Additionally, they contain a variety of vital minerals, including manganese, copper, iron, potassium, and calcium. India is the next-largest producer of peppermint and spearmint, followed by the United States of America (Akter et al., 2016). There are around 25 species of mint, each with a different level of ploidy. Peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), wild mint (*Mentha arvensis*), pennyroyal (*Mentha pulegium*), and bergamot mint (*Mentha citrate*) are the five main species of mint (Tuly et al., 2015).

Because of their anti-feeding, insecticidal, antiviral, antibacterial, immuno-modulating, and anti-aging effects, peppermint and spearmint are the most commercially successful mint species. Tea, drinks, jellies, syrups, candies, and ice cream all contain mint leaves. Typically, *M. piperita* and *M. arvensis* leaves are used to make peppermint oil. A variety of foods and medications include menthol (Tuly et al., 2015). All mints include menthol, a volatile substance that gives mint its distinctively calming, purifying sensation. The therapeutic qualities of mint are numerous. It serves as an antacid, air freshener, mouth freshener; aids in digestion, calms frayed nerves, and may even treat cancer. Mint is one of the most adaptable plants in the herbal kingdom thanks to its distinctive perfume, savory flavor, refreshing sensation, and medicinal properties. Body lotions, soaps, bath oils, and skin tonics all use mint oil as an ingredient. Applying mint oil helps to calm the skin's surface and clear it of blemishes (Hoque & Akter, 2014). Leaves can be used to make herbal tea, which has been traditionally used to treat a number of minor ailments like fever, headaches, and digestive disorders (Rahman et al., 2013).

1.1.6. Onion

Onion (*Allium cepa*. L.) belongs to Liliaceae, which is native to central Asia, is one of the oldest plants that have been cultivated; it has been grown there for more than 4,000 years. There are 80 species, with the majority found in the western US states and Mexico and Guatemala (Boonerjee et al., 2006a). Although most successfully between 7° and 27°C, onion seeds germinate best at 15.5°C (Boonerjee et al., 2006b).

Due to its aroma and flavor, it is used fresh as a powder, an essential oil and a spice to enhance food flavor. There are numerous components in *A. cepa* and demonstrates a variety of pharmacological traits. Ancient Egypt is where *A. cepa* was first used because of its healing, anti-inflammatory, and anti-microbial properties. It has also been acknowledged as a successful treatment for hepatitis, throat infections, and stomach ailments since antiquity. *A. cepa* tea is used in Chinese medicine to treat fever, headaches, cholera, dysentery, the common cold, and arthritis. The improvement of kidney function, as well as anthelmintic, aphrodisiac, carminative, emmenagogue, and expectorant activities, have been associated with this plant's pharmacological properties. Additionally, it is effective for treating bruises, earaches, jaundice, pimples, wounds, scars, keloids, vertigo, fainting, migraine, pain and swelling after bee or wasp stings. (Marefati et al., 2021).

Table 3 *Pharmacological Activities of Allium cepa. L. (Source: Marefati et al., 2021)*

Sl. No.	**Pharmacological Activities**
1	Antifungal
2	Anticancer
3	anti-inflammatory
4	Antioxidant
5	Antispasmodic
6	Antimicrobial
8	Antidiabetic
9	Antimicrobial
10	Antithrombotic
11	Antitumor
12	Anti-hyperlipidemic
13	Anti-arthritic
14	Anti-hyperglycemic anticarcinogenic

1.1.7. Mustard

A significant oil-producing crop in the Brassicaceae family is Brassica. *Brassica* is the third-ranked vegetable oil crop in the world, after soya bean oil and palm oil. It is the second most popular protein meal source. *Brassica* seeds provide 20–25% protein and 40–45% oil. Due to the presence of omega 3 and fatty acid compositions, it is one of the best cooking oils, especially for heart patients (Dina et al., 2019).

The vegetable and oilseed crops in the genus *Brassica* are significant commercial crops and a valuable source of nutrients and phytochemicals that promote health. High consumption of these crops lowers the risk of numerous types of cancer as well as age-related chronic disorders like cardiovascular health and other degenerative diseases (Sarker et al., 2016). One of the most significant sources of industrial oil, protein-rich products, and edible vegetable oil in the world is oilseed Brassica. A lot of the oil's characteristics have a considerable positive impact on human health (Goswami et al., 2020).

1.1.8. Turmeric

As a perennial plant with a short stem and broad, oblong leaves of the Zingiberaceae family, *Curcuma longa*, from whose rhizome's turmeric is produced, bears oblong, pyriform, or oblong rhizomes, which are typically brownish-yellow in color and branching. Turmeric a native of South-East Asia, is used as a food additive (spice), preservative and coloring agent in Asian countries including China, Bangladesh and South East Asia. It is primarily cultivated in China, Taiwan, Sri Lanka, Bangladesh, Burma (Myanmar), Nigeria, Australia, West Indies, Peru, Jamaica and some other Caribbean and Latin American countries. India is the world's greatest producer of turmeric, making up roughly 78 percent of global output. Turmeric is also the most widely used and exported commodity there (Rathaur et al., 2012).

Use of turmeric stretches backs nearly 4000 years to the Vedic civilization in India. It is widely utilized as a natural treatment for many ailments in Ayurveda, Unani, and Siddha medicine. It is often used in traditional Hindu medicine to alleviate sprains and swelling brought on by injuries. Turmeric powder has recently been used in traditional Indian medicine to treat conditions such as biliary disorders, anorexia, coryza, cough, diabetes, wounds, hepatic disorders, rheumatism and sinusitis etc. (Rathaur et al., 2012). Recent research has shown that turmeric can help with oral health. It serves as a natural yellow coloring agent, a component of perfumes, and a permitted food additive. As a part of curry, curcumin is frequently consumed. An orange-yellow crystalline powder known as curcumin has been linked to potential health advantages for diabetes, cancer, and several inflammatory illnesses including arthritis and cardiovascular disease (Singletary, 2020).

1.1.9. Tulsi

For its therapeutic potential, the Lamiaceae genus *Ocimum* is the most significant medicinal plant and spice. It is commonly referred to as holy Basil, Tulsi, or Tulasi and grows in a low bush up to 45 cm tall. Tulsi is revered as an "elixir of life" that has no equal for both its healing and profound qualities and is known in Ayurveda as "The Incomparable One," "Mother Medicine of Nature," and "The Queen of Herbs" (Jamal et al., 2016). *Ocimum tenuiflorum* (Tulsi), *Ocimum gratissium* (Ram Tulsi), *Ocimum canum* (Dulal Tulsi), and Ocimum americanum are the important *Ocimum* species (Ban Tulsi). The most significant herbal plant among them, *O. tenuiflorum* (also known as *O. sanctum* L.), is employed in religious and cultural rituals, folk medicine, and other traditional uses (Akter et al., 2020).

Our daily lives are greatly impacted by tulsi. Due to the numerous medical benefits, it offers (**Table 4**), the tulsi plant is very important to humanity. However, it is also ingested as herbal tea. Tulsi, on the other hand, is a common ingredient in Thai cooking, particularly when making a stir-fry dish with meat or seafood. Across all continents, it is used as a flavoring in a variety of foods, from pizza and cheese to vegetables and sauces. Tulsi is a common ingredient in Thai food as well. It is used to make pesto and can be added to salads, desserts, stir-fries, tea, and other dishes.

Table 4 *Health benefits of Tulsi (Source: Kumar et al., 2010)*

Sl. No.	Pharmacological Activities	Sl. No.	Pharmacological Activities
1	Anti-inflammatory	10	Digestive
2	Anti- stress	11	Anti-aging
3	Anti-bacterial	12	Anti-allergic
4	Anti-oxidant	13	Anti-cancer
5	Analgesic	14	Stimulant
6	Immune modulators	15	Anti-diabetic
7	Carminative	16	Anti-fungal
8	Cardiac toner	17	Antipyretic
9	Beautifying	18	Blood purifier

1.2 National status of some common spices

Bangladesh's cuisine system traditionally relies heavily on spices. Bangladesh's population is expanding quickly, consumer habits are changing, and there are many new methods to incorporate spices into different foods, which are driving up demand for spices in the country. As the area and production of spices increase, the nation will need to import roughly 4000 core BDT in foreign spices to meet its annual need (Rana et al., 2021). In Bangladesh, the main subsistence agricultural method was based on spices. 109 different types of spices are currently grown around the world, but only 27 are consumed and 17 are produced in Bangladesh (Huda et al., 2008) 3.96 lakh hectares in Bangladesh are used for growing spices, with a total yearly production of 24.88 lakh metric tons and a 30 lakh metric tons demand for the seeds. The disparity between supply and demand is likewise widening. Numerous research institutions are working to increase the quantity and caliber of spices. In the past 20 years, BARI has refined and released 18 (major-12, minor-6) disease-resistant spices (Nahar, 2017).

The largest onion growing regions in Bangladesh include Madaripur, Manikganj, Jhenaidah, Khulna, Khustia, Meherpur, Pabna, and Rajshahi. In terms of production, it was ranked top among all other spice crops in Bangladesh, producing over 17,38,000 metric tons of onions between 2017 and 2018. The adoption of many high yield varieties, such as Taherpur and Faridpur Bati, BARI Piaz -1 (1996), BARI Piaz -2 (1999), BARI Piaz -3 (2000), BARI Piaz -4 (2008), and BARI Piaz -5 (2008), has resulted in an increase in onion production due to area growth (Rana et al., 2021).

Bangladesh is a successful producer of black pepper. The spice has a significant chance of making rich foreign exchange through export because it is still in high demand in industrialized nations (Rahman, 2006). It is primarily grown in Moulvibazar, Sylhet, Bangladesh, where it is used to make herbal medicines and as a spice **(**Khan et al., 2017).

Commercial garlic farming is practiced in a number of Bangladeshi areas. However, Madaripur, Rajbari, Natore, Pabna, Rajshahi, and Dinajpur are the principal garlic-producing regions in Bangladesh, and in 2017–2018, over 4,62,000 metric tons of garlic were produced there across an area of 71225 hectors. The introduction of new, enhanced garlic varieties, such as BARI Rashun-1, BARI Rashun-2, BAU Rashun-1, and BAU Rashun-2, has resulted in an increase in area and production while also providing farmers with a higher income. BARI Rashun-1 and 2 outperformed traditional varieties in terms of productivity by 45 and 56 percent, respectively (Rana et al., 2021).

Mint is widely grown throughout Bangladesh, but at the moment, mint oil is imported from other countries to be used in industries (Tuli et al., 2015)**.** Despite its enormous significance and demand across a variety of cosmetic and pharmaceutical businesses as well as from consumers, there are no statistics on the production of mint in our nation (Hoque & Akter, 2014). According to representatives of the Department of Agricultural Extension (DAE), for the past few years, 200 farmers from various villages in the following five districts have been growing mint for a living: Lalmonirhat, Kurigram, Gaibandha, Nilphamari, and Rangpur. The annual production of mint by each commercial farmer ranges from 50 to 60 kilograms (kg) per decimal, with the plant fetching prices of Tk 150 to Tk 200 per kg. (Roy, 2022)

One of the most widely grown spices in Bangladesh is chilli. Faridpur, Rajbari, Pabna, Rajshahi, Bhola, Shariatpur, and Panchagarh are Bangladesh's top chili farming regions. Bangladesh produced 54630 metric tons of chili on an average area of 79180 ha, with a yield of just 0.69 metric tons per hectare. The development and propagation of better varieties as well as the widespread use of BARI Morich-1 have increased the productivity of chili. In contrast to the local chili line, BARI Morich-2 produced the most fruit with the longest sizes (Rana et al., 2021).

Tulsi is the most sacred plant in Bangladesh. Due to the reasonable costs of the herb leaves, tulsi (a medicinal plant) cultivation is progressively gaining favor among farmers in many villages throughout the district. Among the villages are Ramchandrapur and Muraripur in the Palashbari upazila, Kunjamohipur and Khordakamarpur in the Sadullapur upazila, Shahpara Bhabanipur in the Sadar upazila, Sreepur and Ghoshpara in the Sundarganj upazila, and Barokhodapur in the Gobindaganj upazila (Correspondent & Gaibandha, 2019)

Ginger grows well in northern part of Bangladesh, such as Nilphamari, Dinajpur, Rangpur as well as other parts, like, Khulna, Rangamati, Bandarban, Khagrachari and Gazipur districts. (Ferdous, 2014). Bangladesh's ginger production rate is not acceptable. However, the introduction of the new, high-yielding ginger varieties BARI Ada-1(2008), BARI Ada-1(2012), and BARI Ada-3 may be the likely cause of the increase in ginger production in the recent years (Rana et al., 2021).

In Bangladesh, rapeseed and mustard are grown on 0.325 million hectares, which is around 60 percent of the total area used for oilseed crop cultivation. Mustard accounts for almost 70 percent of Bangladesh's total oil production. Bangladesh produces roughly 0.254 million tons of oil seeds, which is about 39 to 40 percent of what the nation needs. Rapeseed and mustard may be grown relatively successfully in Bangladesh due to its good climatic and environmental conditions. The Bangladesh Institute of Nuclear Agriculture (BINA) has created and made available three different strains of *B. campestris*: var. Agrani, BINA Sarisha-10, and BINA Sarisha-6. These variants have some distinctive qualities. The mustard variety with yellow seeds and a thick pod coat is called Agrani. Black seeded mustard cultivar BINA Sarisha-10 matures quickly. Yellow seeded BINA Sarisha-6 is a salt tolerant (up to 13 dS/m) cultivar. All three types' seeds have an oil content of 42–44 percent (Goswami et al., 2020).

Bangladesh is the fifth-largest producer of turmeric worldwide (Rahman, 2020). Khulna Division ranked first in Bangladesh for annual output of turmeric in 2018–19, accounting for

32.52 percent of the country's total production followed by Rajshahi (Major turmeric producing, n.d.).

1.3 Plant tissue culture

The most recent approach in the field of cell biology is plant tissue culture (Hossain, 2018). Micropropagation is a special tool that is particularly useful for retaining heterozygous genotypes and maintaining the genetic uniformity of the best plant materials (Akther et al., 2020). Plant tissue culture, which includes micropropagation, induction of somaclones, somatic hybridization, cryopreservation, and regeneration of transgenic plants, is the foundation of plant biotechnology. Any plant portion can be cultivated using the technique of "plant tissue culture" on a sterile nutritional medium under controlled lighting and temperature conditions in order to produce growth. Plant tissue culture was conceptualized as a result of Schwann's cell theory, which he developed in 1839. For many years, tissue culture methods have been crucial in the micropropagation of horticultural and ornamental plants. In actuality, horticultural plants were used to produce the first ever successful plant tissue culture. These methods have been widely employed in the control of illness and vegetative reproduction (Ferdous, 2014).

1.4 Importance of plant tissue culture

Tissue culture has proven to be a crucial technique in biotechnology since it provides similar quantities of plant components with protection against plant disease. It is necessary to create a protocol for plants *in vitro* propagation in order to obtain healthy, disease-free plant materials. The usual clonal propagation approach seems insufficient to meet the expanding demand for disease-free and healthy plants; hence an alternative strategy for crop enhancement has long been called for. Plant tissue culture enables the preservation of plant germplasm, pathogen-free production, and rapid replication. Techniques for tissue culture may be essential in this situation (Mamun, 2006).

When compared to other methods, *in vitro* techniques for the preservation and application of plant germplasm can have some clear advantages (Ferdous, 2014).

These are a few of them:

1. Through meristem culture, it may be possible to remove viruses from infected tissue.
2. Where producing clonal material is necessary to maintain superior genotypes,
3. Micro propagation techniques can be used at any moment when stocks are needed to multiply quickly.
4. Breeding programs may benefit from facilitating the germination of challenging or immature seeds or embryos, and
5. Regarding the health status of the germplasm when distributed across the border utilizing *in vitro* cultures, this may be safer.

The fact that *in vitro* procedures demand significantly less storage space than field storage is one of its many more all-encompassing favorable benefits. Anywhere in the world can set up storage facilities, and cultures are not affected by environmental disturbances such as temperature changes, cyclones, insects, pests, and pathogens (Ferdous, 2014). Consequently, *in vitro* tissue culture followed by gene transfer may be a simple, effective, and affordable way to quickly produce a large number of disease-free, reliably uniform, and true-to-type plants that will increase production and quality (Ashrafuzzaman et al., 2009). On the other hand, traditional crop development techniques are insufficient to deal with the growing population, diminishing land resources, and rising environmental stress. Any enhancement to a crop requires genetic variety. The only practical method for producing a large number of clonal plants quickly is bulk tissue culture propagation of uniform, healthy plants.

1.5 Objectives

Objectives of the current study are:

1. Study the current *in vitro* research state of spices researches in Bangladesh.
2. Compare the micro propagation methodologies reported in Bangladesh for spices.
3. Identify the cause of variation in *in vitro* regeneration

Chapter 2

Materials & Methods

Chapter 2

Materials & Methods

2.1 Data selection

“Spices”, “*In vitro*”, “regeneration” “micropropagation” and “Bangladesh” were used as the key words in Researchgate, ScienceDirect, Springer, Google scholar and PubMed searches for both original papers and reviews. A few references were also obtained from the references list of the articles that were reviewed. The journals that were accessed to retrieve articles were Bangladesh Association for Plant Tissue Culture & Biotechnology, Journal of Agriculture and Food, BanglaJOL etc. Articles were also collected from websites of different universities across the country and several research institutes such as Bangladesh Agricultural Research Institute, BINA, BCSIR, etc.

2.2 Study selection

Articles were chosen, on the basis of how relevant they were to the present study objectives. The secondary sources were the only ones from which the data were collected. I gathered important data for this article while researching it in a variety of journals, reports, publications, etc. Additionally, I looked up relevant research organization websites and prominent lab websites on the internet to gather information. After that, I put together and wrote this review paper after gathering all the information that was available.

Chapter 3

Results & Discussions

Chapter 3

Results & Discussions

Nowadays, to enhance spice production and quality, numerous research institutions are involved. In Bangladesh, various government and non-government organizations as well as educational institutions are active in this field. For the development of spices, many plant breeders have been using biotechnological methods. But in Bangladesh, there are very few of them. Some of the most relevant research from previously completed related works by various Bangladeshi institutions have been studied and are cited here.

3.1 Onion

3.1.1 Onion result

Onion (*Allium cepa* L.) tissue culture hasn't been the subject of many reports from Bangladesh. Only two articles from Bangladesh were found which deals with *in vitro* regeneration of onion. Both of the research was conducted at Khulna University and achieved direct regeneration using MS media, with different combinations and concentrations of hormones. The same research group carried out both the experiments.

Explant

For both the papers shoot was used as explant. Boonerjee et al., (2006a) used shoot tips and Boonerjee et al., (2006b) used shoot clumps as explant.

Shoot regeneration

Both of the experiment used the combination of BAP and NAA to get the best response in shooting. Boonerjee et al., (2006b) only used BAP in combination with NAA whereas Boonerjee et al., (2006a) used BAP + NAA for shoot initiation and later used BAP + 2ip for shoot manipulation.

Root regeneration and survival rate

Each paper used different hormones for rooting. Boonerjee et al., (2006a) reported of using 2.0 mg/l IBA for both the cultivars which gave rooting of 90 percent on Taherpuri and 80 percent on Indian variety. However, Boonerjee et al., (2006b) used MS containing 1.0 mg/l 2ip which gave about 75 percent of Taherpuri and 60 percent of Indian plantlets survivability rate.

3.1.2 Onion discussion

In shoot tip culture of *Ocimum sanctum* L., Begum et al., (2000) discovered that 0.1-1.0 mg/l NAA worked best for root induction. Both of the experiment from Bangladesh is in disagreement with above mentioned findings, as IBA and 2ip gave the best response in rooting.

Both of the papers worked with same varieties of onion. But they used different concentration and combinations of hormone. This demonstrates that different hormonal combination and concentration plays a role in *in vitro* regeneration. It can be concluded with the fact that different concentration and combinations of hormones that has been used in both experiments are efficient and successful for the clonal propagation of onion and can be used for further studies.

3.2 Black pepper

3.2.1 Black pepper result

Not much report is available on black paper (*Piper nigrum* L.) tissue culture from Bangladesh. There are only two articles for *in vitro* regeneration on black paper from Bangladesh. One of them conducted direct regeneration and the other conducted indirect regeneration. Each article worked with different explants and different combination and concentrations of hormones.

Explant

Two articles used two different explants for their experiment. Nodal explant was used by Khan et al., (2017), whereas cotyledon was used by Rahman (2006). Because different regeneration systems were used, there can't be any comparisons.

Shoot regeneration

IAA was crucial in the production of black pepper shoots for both direct and indirect regeneration. Rahman (2006) used IAA along with BA to produce callus. Later he used the same combination of hormones to produce shoots. Khan et al., (2017), on the other hand, used a direct regeneration protocol and combined IAA with BAP to produce shoots.

Root regeneration and survival rate

IBA has also been found to be an important hormone in rooting. To produce roots, both articles used 1.5mg/l IBA. However, Khan et al., (2017) mentioned the supplementation of half MS media. This hormonal treatment gave Khan et al., (2017) 95 percent survival rate while Rahman (2006) achieved 88 percent.

3.2.2 Black pepper discussion

Mathews and Rao (1984) worked with the same concentration and combination of hormones as Khan et al., (2017) for shoot formation of *Piper nigrum* L. However, they used shoot tips as explant. From Bangladesh Rahman et al., 2006 reported that MS media containing BA+IAA produced callus which is green in color and the media containing only BA produced light green color callus. Sari et al., (2018) reported that the appearance of green color in callus indicates the presence of steroids. The greener color callus indicates more chlorophyll content, which can support the growth of the callus. The color variations in callus could be due to different types of growth regulators, the difference in growth regulator concentration and combination, and the type of explant. Afshari et al., (2011) stated that various callus color conditions could be caused by the pigmentation, the influence of light, and the plant parts used as the source explant.

3.3 Chilli

3.3.1 Chilli result

From Bangladesh, there were only three researches available on chilli micropropagation. Although all three used MS media, the hormone combinations and concentrations used are varied. Additionally, they used a variety of explants.

Explant

Each experiment used different explants, such as hypocotyl, cotyledon, shoot tip, cotyledonary leaf, and node. In 2009, Ashrafuzzaman and his colleagues conducted both indirect and direct regeneration of chilli. Where for indirect regeneration he used hypocotyl and cotyledon and for direct shoot tip was used. He reported that the hypocotyl outperformed the cotyledon in terms of performance.

Shoot regeneration

Hossain (2018) and Ashrafuzzaman et al., (2009) both conducted indirect regeneration in their experiment where they both used BAP but with another hormone to induce callus. While using hypocotyl as explant Ashrafuzzaman et al., (2009) used BAP along with NAA and Hossain (2018) used BAP in combination with IBA on shoot tip explant. In all the experiments, BAP was frequently combined with other hormones to promote shoot regeneration. Ashrafuzzaman et al., (2009) used BAP in combination with $AgNO3_3$, Hossain (2018) used BAP along with NAA and IAA and Akther et al., (2020) used BAP in combination with IBA. As different hormones and explants were used, each experiment provided different percentage of shoot regeneration.

Root regeneration and survival rate

IBA has been found to be a crucial hormone for rooting. Ashrafuzzaman et al., (2009) found IBA in combination with NAA to be most effective for rooting. On the other hand, Hossain (2018) and Akther et al., (2020) used IBA alone. Akther et al., (2020) used MS with IBA (0.5 mg/l) which gave survivability of 85 percent in red variety and 55 percent in yellow variety. In contrasted to that Hossain (2018) used 2.0 mg/l IBA which gave survivability of 75 percent in natural condition.

3.3.2 Chilli discussion

Variation in callusing, shooting, rooting was observed in all three experiments. Environmental factors can have a significant impact on the establishment of cultures and subsequent plant regeneration, as well as the age, nature, origin, and physiological state of the explant, which all contribute to this variation. All of the experiments from Bangladesh used different PGRs for rooting. However, Song et al. (2010) employed MS without PGRs to root the 'Hivita Red' and 'Hivita Yellow' varieties of *C. annuum*.

Further research on pepper should be continued, as this micro-propagation technique of pepper is still not so well established in Bangladesh.

3.4 Garlic

3.4.1 Garlic result

There was a total of four articles on the regeneration of garlic *in vitro* that came from Bangladesh. Two of them involved indirect regeneration, while the other two focused on direct regeneration. Although all four used MS media, the hormone combinations and concentrations used varied.

Explant

The most frequently used explant was the root tip, followed by the basal disc, the leaf base of the sprouted garlic cloves, and the base of the clove. In 2015, Haider and his colleagues conducted indirect regeneration and worked with 3 different explants where he found basal disc to superior over root tips and leaf base of the sprouted cloves of garlic.

Shoot regeneration

To regenerate callus and shoots, various hormones were used in a variety of combinations. This led to various results. Some studies suggested using a single hormone, while others suggested combining hormones. For maximum callus induction Hassan et al., (2014) and Haider et al., (2015) both used 2,4-D, but Haider et al., (2015) used 2,4-D in combination with BAP where Hassan et al., (2014) used 2,4-D alone. For shoot regeneration Haider et al., (2015) and Parvin et al., (2007) used Kn and BAP in combination. On the other hand, Mubarrat et al., (2018) and Hassan et al., (2014) used Kn alone. Each hormonal combination gave promising results in shoot regeneration. However, the outcomes were not uniform. This could occur as a result of the utilization of different genotypes.

Root regeneration and Survival rate

Similar to shooting, each paper utilized a unique hormone combination and concentration for rooting. However, Haider et al., (2015) and Hassan et al., (2014) did not report anything regarding rooting in their experiment. Mubarrat et al., (2018) reported of 100 percent rooting in combination of 2.5 mg/l Kn + (1.0, 1.5) mg/l 2, 4-D. 86 percent survivability rate was found on this combination in field condition. On the other hand, Parvin et al., (2007) suggested that 1.0 mg/l NAA and 4.0 mg/l BAP will result in 75 percent survivability rate.

3.4.2 Garlic discussion

The papers discussed different garlic genotypes (G121, G122, G123 and G124, Chinese verity, Japanese cv. white roppen, and Bangladeshi garlic). Different explants performed differently depending on the genotype variation. Variations in callusing, shooting and rooting were caused by different hormonal concentrations and combinations. Growth regulators in the culture medium, as well as genetic, physiological, and morphological changes in the *in vitro* environment, may be to blame for this variation.

Haider et al., (2015) observed callus induction better in 2,4-D along with BAP response. On the other hand, as an alternative to BAP as a cytokinin source, Khan et al., (2004) found a higher percentage of callus induction in MS medium supplemented with 2,4-D and Kn. Chinese cultivars outperformed other cultivars according to Parvin et al., (2007) who reported best performance for shoot induction from root-tips by NAA and BAP. Similar findings were reported by Haque et al., (2000), who found that using NAA and BAP, Japanese and Chinese cultivars outperformed other genotypes.

3.5 Ginger

3.5.1 Ginger result

Bangladesh has produced a significant amount of ginger by micro propagation. Both the direct and indirect regeneration protocols were followed in this instance. A total of five articles from Bangladesh have been discovered, of which four used a direct regeneration technique and one an indirect regeneration protocol. Although all five employed MS medium, the hormone combinations and concentrations, as well as the explants, varied.

Explant

Among all the papers shoot tip was the mostly used explant followed by rhizome buds, sprout, leaf and root. In 2016, Sumon and his colleagues worked with both rhizome buds and shoot tips, where he found rhizome buds to outperform shoot tips. Sultana et al., (2009), on the other hand, used a leaf, root, and shoot tip and discovered that the leaf performed much better than the root and shoot explant. The aforementioned variance could be brought about by the use of several growth regulators as well as genetic, physiological, and morphological variables.

Shoot regeneration

To regenerate callus and shoots, various hormones were used in various combinations. Sultana et al., (2009) conducted indirect regeneration and he demonstrated that MS media containing 0.5 Dicomba on the leaf explant of Suruchi verity is promising for callusing. For shooting most of the papers used the combination of BAP and NAA or BAP or Kn. Sumon et al., (2019) used all BAP, Kn and NAA to produce shoots. On the other hand, both Yesmin et al., (2016) and Mamun (2006) used BAP and NAA and found promising results. Contrast with that Sultana et al., (2009) used BAP and Kn in combination for shooting. Ferdous (2014) went with a different approach and used GA_3 in combination with BAP to produce shoots.

Root regeneration and Survival rate

In the instance of rooting, both a single hormone treatment and a combination of treatments were detected. IBA was used in all five papers either alone or in combination with NAA. Yesmin et al., (2016), Ferdous (2014) and Sultana et al., (2009) used IBA alone and got promising result in rooting. However, Yesmin et al., (2016) used half MS media in this process where 85 percent plants survived. Ferdous (2014) also informed to have 86 percent survival rate in pot condition. On the other hand, Sumon et al., (2019) and Mamun (2006) both used IBA in combination with NAA where Sumon et al., (2019) found survivability rate of 95 percent and 85 percent in BARI Ada-1 and Chinese ginger, respectively.

3.5.2 Ginger discussion

The papers dealt with different genotypes of ginger (BARI Ada-1, Chinese ginger accession number SG876 and Suruchi). Different outcomes were observed in all five experiments. Sumon et al., (2019) demonstrated that rhizome bud explant and BARI Ada-1 variety performed better in his experiment. This could be due to genetic, physiological, and morphological variation *in vitro* as well as various growth regulators in the culture media.

From Bangladesh Sumon et al., (2019) observed highest shoot response on ginger rhizome bub with MS supplemented with 2.0 mg/1 BAP and 0.5 mg/1 Kn. However, Khatun et al., (2003) stated the best shoot response came on MS with 2.5 mg/1 BAP and 0.5 mg/1 Kn in ginger shoot tips. In the observation of Sultana et al., (2009), is demonstrated that for callusing explant leaf performed better than root. This finding supports the findings of Babu et al., (1992).

3.6 Turmeric

3.6.1 Turmeric result

Despite having a high consumption rate of turmeric in Bangladesh, very little research has been done on *in vitro* regeneration of turmeric. Only three researches can be found on this topic. All three conducted direct regeneration protocol; however, they employed various hormone combinations and concentrations as well as various media.

Media

Among the three experiments, uses of two different media can be seen. Rahman et al., (2004) and Sharmin et al., (2013) worked with MS media, on the other hand Nasirujjaman et al., (2005) used WPM as medium.

Explant

All the experiment used rhizome bud as explant. However, each experiment produced different results. This could be due to the supplementation different hormonal concentration and combinations.

Shoot regeneration

Different hormones were employed in different combinations to regenerate shoots. Rahman et al., (2004) and Sharmin et al., (2013) used single hormone BA supplemented with MS media, where Rahman et al. (2004) found 100 percent shoot proliferation on 2.0 mg/l IBA and Sharmin et al., (2013) found 95.3 percent shoot proliferation on 1.0 mg/l BAP. On the other hand, Nasirujjaman et al., (2005) a combination of BAP and NAA supplemented with WPM, which gave 95 percent shoot proliferation.

Root regeneration and Survival rate

Both a single hormone treatment and a combination of treatments were found in the case of rooting. Both Rahman et al., (2004) and Sharmin et al., (2013) found 100 percent rooting though they used different hormonal concentrations and combinations. Rahman et al., (2004) used half strength of MS medium containing 2.0 mg/l IBA resulting in 70 percent survivability of the regenerated plantlets. Contrasted with that, Sharmin et al., (2013) used NAA at 0.5 mg/l and reported 85 percent plantlets survival. There was no report of rooting in the experiment of Nasirujjaman et al., (2005).

3.6.2 Turmeric discussion

The papers discussed two different types of turmeric (*Cucurma longa Linn., Curcuma aromatica,*). Despite the similarity of the explant in all the articles, the results varied since different hormone concentrations and combinations were used. Also, this diversity could be caused by the explant's age, origin, and physiological state. Environmental factors can also be extremely important for the development of cultures and subsequent plant regeneration.

Nasirujjaman et al., (2005) from Bangladesh reported shoot induction by using WPM 4.0 mg/l BAP + 1.0 mg/l NAA. Similarly, Sunitibala et al., (2001) demonstrated clonal propagation of turmeric using 2.0 mg/l BAP + 1.0 mg/l NAA where she used MS media.

Further research on turmeric should be continued, as there’s not much work that has been done yet.

3.7 Tulsi

3.7.1 Tulsi result

There wasn’t much research reported from Bangladesh on tulsi tissue culture. On the *in vitro* regeneration of tulsi from Bangladesh, there are only two articles available. Direct regeneration was carried out in both articles using MS media and various hormone combinations and concentrations.

Explant

In both the experiments, nodal segments were used as explant. However, Jamal et al., (2016) used both nodal segments and shoot tips, with nodal segments outperforming shoot tips in terms of performance.

Shoot regeneration

In 2020, Akter and her coworkers studied two different tulsi varieties and discovered two distinct media combinations that were best for their respective varieties. BAP was found to deliver the best response in shooting in combination with Kn and NAA for *Ocimum tenuiflorum* and *O. americanum*, respectively. On the other hand, Jamal et al., (2016) discovered, BAP in combination with NAA resulted best in shooting response in *Ocimum sanctum* L. Therefore, in both the report combination of cytokine with auxin seems to give good shooting.

Root regeneration and survival rate

For rooting Akter et al., (2020) used half MS media for both of the variety whereas Jamal et al., (2016) used full MS media. IBA and NAA gave the best performance in root induction for *Ocimum tenuiflorum* and *O. americanum*, respectively. This treatment provided 80 percent survival rate for both of the varieties. Jamal et al., (2016) on the other hand, achieved best rooting response using NAA in full MS medium.

3.7.2 Tulsi discussion

These papers covered different varieties of tulsi (*Ocimum tenuiflorum*/ *Ocimum sanctum* L., *O. americanum*). According to Jamal et al., (2016), nodal segments outperformed shoot tips among the two explants. Akter et al., (2020) found a good response for shooting when using BAP in with IAA. The report by Susila et al., 2013 also demonstrates that higher concentration of BAP results in multiple shoot formation (Susila et al., 2013).

The purpose of both of the studies was to establish an efficient *in vitro* regeneration protocol for tulsi and to identify the best explant. In this species, both of the protocols are completely applicable for a wide range of tasks, including *in vitro* conservation, cryopreservation, significant scale augmentation, and hereditary transformation.

3.8 Mint

3.8.1 Mint result

Quite a few studies have been reported on the micropropagation of different varieties of mint have been found in Bangladesh. Each paper worked with different genotype, explant and different combination and concentration of hormones. However, all of them conducted direct regeneration protocol and used MS media.

Explant

For explant, nodal segment and shoot tip were the most commonly used explants followed by leaf. Majority of the experiments suggested nodal segment and shoot tip over leaf. In 2016 Akter and her colleagues found shoot tip and nodal segments to perform better over leaf. Similarly, Akter & Hoque (2018), Tuly et al., (2015) and Hoque & Akter (2014) found resembles in their studies with this finding. It can be suggested, working with shoot tip and nodal segments as explants may provide the best chance of achieving maximum success.

Shoot regeneration

Different hormones, in various combinations, were used to regenerate shoots. This produced different outcomes. Some studies recommended using a single hormone, while others recommended using hormones in combination. Both Akter & Hoque (2018) and Islam et al., (2017) worked with only BAP to produce shoots. However, Akter & Hoque (2018) used 1.0mg/l BAP while Islam et al., (2017) used 3.0 mg/l BAP and obtained 100 percent frequency. Ahmed (2018) on the other hand, used 2.0 mg/L BA and achieved 80 percent shoot induction from shoot tip. In case of combination treatments, MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l IAA was used by Rahman et al., (2013). On the contrary, Tuly et al., (2015) and Hoque & Akter (2014) used the same concentration and combination of hormones, (1.0 mg/l NAA+ 1.0 mg/l BAP), but the results varied. This might be due to the use of different genotypes.

Root regeneration and Survival rate

Similar to shooting each paper used different concentration and combination of hormones along with half and full-strength MS media. Akter et al., (2016) and Islam et al., (2017) used only IBA for rooting. Akter et al., (2016) demonstrated that full strength MS media with 1.o mg/l IBA gives the best rooting, where Islam et al., (2017) suggested 1.5 mg/l IBA as they received 85 percent rooting from this hormonal supplementation which gave 100 percent survival rate. Ahmed (2018) observed that BA 2.0 mg/l + IBA 1.0 mg/l gave 85 percent rooting on shoot tip explant. This experiment gave 90 percent survival during in growth chamber and 83.33 percent in shade house and 80 percent in open atmosphere at direct sunlight. On the other hand, Rahman et al., (2013) demonstrated that using half strength media with 1.5 mg/l IAA will result in 100 percent rooting and 90 percent survival rate. Another study Hoque & Akter (2014), using the same hormonal combination and concentration as they used in shooting (1.0 mg/l NAA+ 1.0 mg/l BAP), resulted in 100 percent rooting.

3.8.2 Mint discussion

The papers dealt with various genotype of mint (MP-1, MP-2, MP-3, MP-4, MP-5, MP-6, MP-9, MP-12, MP-19 and MP-22). Among the three explants, node and shoot tip performed better than leaf. The presence of an apical bud in those explants may explain their superior performance. Variation in species and different plant organs could also be factors. Different hormonal concentration and combination produced variation in shooting. This variation could be caused by growth regulators in the culture media, as well as genetic, physiological, and morphological changes in the *in vitro* environment. Similarly, different hormonal concentration and combination also gave different results in rooting. Islam et al., (2017) reported that in 1.5 mg/l IBA, the most roots were produced. *Mentha viridi*s also produced a similar result which was demonstrated by David & Arockiasamy (2008). According to Heidari et al., (2012), shoot meristems and nodes were more effective for regenerating new shoots than leaf disk explants, which was in agreement with the findings of Akter & Hoque (2018).

3.9 Mustard

3.9.1 Mustard result

In vitro regeneration of various mustard (*Brassica* spp.) varieties has been reported in eight articles from Bangladesh. Seven of them performed indirect regeneration and one performed direct regeneration. Each study used a different genotype, explants, as well as a different hormone combinations and concentrations. However, all of them used MS media as basal media.

Explant

The most commonly used explants were cotyledon, hypocotyl, and buds, followed by leaf. Dina and her colleagues worked with cotyledon and hypocotyl as explants in 2019 and discovered that cotyledon outperformed hypocotyl. The same explants were used by Sarker et al., (2016), Alam et al., (2013), and Goswami et al., (2020). On the other hand, Sayem et al., (2010), Alam et al., (2009) and Halim et al., (2013) used flower buds as explant. H. Basak et al., (2012) were the only ones who used leaf as an explant.

Shoot regeneration

All of the articles that performed indirect plant regeneration used a combination of hormones to induce callus. The majority of callus-inducing articles combined BAP with another hormone. NAA and 2,4-D was also commonly used. Sarker et al., (2016) induced a promising percentage of calluses using 1.0 mg/l BAP and 0.5 mg/l NAA. Similarly, Sayem et al., (2010) and H. Basak et al., (2012) also used NAA but in combination with NAA Sayem et al., (2010) used BAP and H. Basak et al., (2012) used 2, 4-D and AgNO3. 2, 4-D was also frequently observed in callus induction. BAP in combination with 2,4-D was used by Alam et al., (2009), Alam et al., (2013) and Halim et al., (2013). Along with BAP and 2,4-D Alam et al., (2013) also used AgNO3 where Goswami et al., (2020) only used 2, 4-D in combination with AgNO3. Despite the fact that Alam et al., (2009) and Alam et al., (2013) wrote specific hormonal concentrations in their papers, the results were found to be anomalous.

To regenerate shoots, various hormones were used in various combinations. All of the articles combined multiple hormones. BAP was used in all of the experiments along with other hormones. Dina et al., (2019), Sayem et al., (2010), Alam et al., (2009) used NAA in combination with BAP. The same treatment was used by Alam et al., (2013), Sarker et al., (2016) and H. Basak et al., (2012), but they added AgNO3. Goswami et al., (2020) and Halim et al., (2013) also used BAP but in the combination IAA and 2,4-D was used respectively. Each treatment produced different results in each article. This could be because of the use of various growth regulators in the culture media.

Root regeneration and survival rate

In the case of rooting, both single hormone treatment and treatment in combination were found. NAA was primarily used for rooting in both single and combination hormone treatments. Dina et al., (2019), Sarker et al., (2016), and H. Basak et al., (2012) used NAA alone to regenerate roots. Where Dina et al., (2019) and Sarker et al., (2016) both achieved 100 percent rooting in MS + 0.1 mg/l NAA and MS + 0.2 mg/l NAA, respectively. H. Basak et al., (2012) showed survival rate 62 percent for Tori-7 followed by 60 percent for BARI Sarisha-9. In contrast to that Goswami et al., (2020) used IBA alone at 0.5 mg/l to regenerate roots giving 80 percent survival rate. Alam et al., (2009) used IBA in combination with NAA. Whereas, Alam et al., (2013), worked with NAA in combination with BAP for rooting.

3.9.2 Mustard discussion

All the varieties (BARI sarisha-8, BARI sarisha-12, BARI Sarisha-6, BARI Sarisha-11, (BARI Shariaha-7, Tori-7, Agrani, Daulat, Safal, Sampad, BARI Sarisha-9, BINA Sarisha-10, SAU sharisha 1, BARI sharisha 15) and explants gave much variation in results in each experiment. Genetic, physiological, and morphological changes in the *in vitro* environment are some major factors for such variation in results, making it difficult to determine which explant and treatment produced the best results. Sarker et al., (2016) suggested that cotyledon performed better in callus induction. Similar findings were reported in Singh S et al., (2011). H. Basak et al., (2012) reported best shoot regeneration on BAP+NAA+AgNO3. The findings of Patil et al., (2006) was also in agreement with this finding.

Chapter 4

Conclusion

Chapter 4

Conclusion

From above findings and discussions, it can be concluded that, each *in vitro* regeneration experiment provided a significant level of variation in their results. The explant's age, nature, origin, and physiological condition all contributed to this variance. As well as due to application of different growth regulators and genetical, physiological and morphological factors are responsible for the variation. Also, the variance can be a result of how environmental elements are influencing it.

Several spice-producing, importing, and exporting nations in the world see significant economic impact from spices and condiments. But unfortunately, the demand for spices in our country is far higher than the supply. Bangladesh may easily enter the export market by manufacturing vast quantities of spices in export quality. But Bangladesh's spice production is not up to par. In Bangladesh, there is no predominant variety. Farmer typically uses regional varieties. Therefore, there is a pressing need to enhance spice production.

Due to the variety of uses for spices, manufacturing is now rising. To enhance spice production and quality, numerous research institutions are involved to meet the demand for spices in our country. However, there are just a few high yielding varieties available. Because of this, the government imports large amounts of spices from foreign nations each year. To solve this issue, we should put a focus on research into spices, create high-yielding, resistant varieties, and employ contemporary methods. There hasn't been enough study done in this area to enhance production. Since spices are a highly valued crop, there is a huge possibility to increase output and make a large profit. Micropropagation might be a key component in finding a solution to this issue.

Recommendations:

- Emphasis in spice research needs to be increased is to boost production.
- Further literature review is needed so that a broad understanding of the in vitro nature of these material can be understood. Applying such findings from other country's research in our field will enhance the regeneration efficiency of our varieties of species.

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