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Soybean Processing Sector in India: Economic and Employment Analysis

R. RAVICHANDRAN AND PREETI DIXIT
Department of Humanities Science Education and Research,
PSS Central Institute of Vocational Education (NCERT), Bhopal, India.

Email: preetidixit07@yahoo.com
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ABSTRACT

India ranks fourth in the soybean cultivation area with 11.34 million hectares (28.02 million acres) accounting for 9.41% of the world soybean cultivation area. India is the world's fifth-largest producer of soybean with 11.22 million tons in 2019-20. Soybean often termed as "golden bean" is one of the major oilseed crops grown in India. Soybean is widely grown in the country as it is considered an important source of protein and oil. In recent years, the demand for soybean products has increased significantly in India due to the rising awareness of health and nutrition among the people. The soybean processing sector in India has been growing rapidly in the last decade, providing ample opportunities for investment and employment generation. This review paper aims to provide an overview of the soybean processing sector in India, its current status, challenges, and opportunities for growth.

Keywords: *Soybean, Soybean processing, Food processing, Government policies, Vocational education, Economic development.*

The soybean processing sector in India is an important and growing industry, with a significant impact on the country's economy. Soybeans are one of the most widely grown oilseeds in India and are used for a variety of purposes, including food, feed, and industrial applications. The soybean processing sector involves the transformation of raw soybeans into a range of products, including soybean oil, soy meal, and other derivative products. The process typically starts with cleaning and sorting soybeans, followed by cracking and dehulling to remove the outer covering. The extracted soybeans are then subjected to oil extraction, either through solvent extraction or mechanical pressing, to obtain soybean oil. The remaining solid material is further processed to obtain soy meal, which is a high-protein ingredient for many industries. The soybean processing sector is characterized by a large number of small-scale processing units, as well as a few large-scale companies. The growth of the soybean processing industry in India has been driven by several factors, including the increasing demand for soy-based products, improvements in technology, and favorable government policies. Despite the growth of the industry, there are several challenges the soybean processing sector is facing in India, including low productivity, lack of standardization, and limited access to financing. In addition, there is a need for greater investment in research and development to improve the quality and competitiveness of soy-based products. Overall, the soybean processing sector in India has a significant potential for growth and development and is poised to play a key role in the country's economy in the coming years.

Market Size and Growth of the Soybean Processing Sector

The market size and growth of the soybean processing industry in India is a complex and dynamic issue that has been influenced by a number of factors. According to recent data, the total market size of the soybean processing industry in India is estimated to be worth around $10 billion, and is growing at a rate of approximately 7% per year. During the last financial year i.e., 2020-21, Soybean processing stood at ~19.8 million tonnes. The global production of soybean has grown at a compound annual growth rate (CAGR) of 2.78% from 315.418 million metric tons in 2015-2016 to 385.524 million metric tons in 2021-2022 (Minhas, A., 2022).

The processing of soybean has been cyclical for 5-6 years One of the key drivers of growth in the soybean processing industry in India has been the increasing demand for soy-based products. This demand has been driven by a growing middle class, as well as the increasing popularity of soy-based products as a source of protein and other nutrients.

Another important factor that has contributed to the growth of the soybean processing industry in India has been the favorable government policies, including subsidies, tax benefits, and other incentives. These policies have helped to encourage investment in the sector and have played a key role in boosting its growth. Overall, the market size and growth of the soybean processing industry in India is a promising sign for the future of this industry and suggests that there is significant potential for further growth and development in the coming years.

Current Status of the Soybean Processing Sector

Soybean production in India has increased from 6.929 million metric tons in 2015-2016 to 11.200 million metric tons in 2021-2022. At the end of fiscal year 2022, India produced over 13.8 million tonnes of soybean reflecting an increase in production from the previous fiscal year (Minhas, A., 2022).

The country has now become the largest importer of soybean oil with an import value of $3.02B in 2020. In the same year, Soybean oil was the 13th most imported product in India (Observatory of Economic Complexity, 2022). Soybean oil is primarily used for food and industrial purposes. The soybean processing industry in India is growing rapidly, with the increasing demand for soybean products in the country. The processing sector includes the production of various soybean-based products, including soybean oil, soybean meal, and other value-added products like soy milk, tofu, snacks item, etc.

The soybean processing industry in India is highly fragmented, with a large number of small and medium-sized companies operating in the sector. The majority of the soybean processing companies in the sector are located in the states of Madhya Pradesh, Maharashtra, and Gujarat. The sector is primarily dominated by large multinational corporations, which have established their production facilities in India to take advantage of the growing demand for soybean products in the country.

Major Players in the Soybean Processing Sector in India

Ruchi Soya Industries Limited: Ruchi Soya Industries Limited is one of the largest soybean processors in India and has a wide range of soybean-based products, including edible oils, dairy alternatives and health foods.

Adani Wilmar Limited: Adani Wilmar Limited is a joint venture between Adani Group and Wilmar International and is one of the largest soybean processors in India. They produce a range of soybean-based products, including edible oils, dairy alternatives and health foods.

Godrej Agrovet Limited: Godrej Agrovet Limited is a subsidiary of Godrej Industries Limited and is one of the largest soybean processors in India. They produce a range of soybean-based products, including edible oils, dairy alternatives, and animal feed.

Ambuja Neotia Group: Ambuja Neotia Group is a conglomerate of companies involved in various businesses, including soybean processing. They produce a range of soybean-based products, including edible oils, dairy alternatives, and health foods.

Agro Tech Foods Limited: Agro Tech Foods Limited is a subsidiary of ConAgra Foods and is one of the largest soybean processors in India. They produce a range of soybean-based products, including edible oils, dairy alternatives, and health foods.

Cargill India Limited: Cargill India Limited is a subsidiary of Cargill Inc. and is one of the largest soybean processors in India. They produce a range of soybean-based products, including edible oils, dairy alternatives, and animal feed.

Emami Agrotech Limited: Emami Agrotech Limited is a subsidiary of Emami Group and is one of the largest soybean processors in India. They produce a range of soybean-based products, including edible oils, dairy alternatives, and health foods.

Market Demand for Soybean Products in India

The market demand for soybean products in India has been steadily increasing in recent years due to its high nutritional value and widespread use in various industries. The growth in the food and feed industries, as well as the demand for plant-based protein, has boosted the demand for soybean products in India. Soybean oil is one of the most commonly used oils in India, while soy flour and meal are widely used as ingredients in the food processing sector and animal feed industry. India's soybean oil imports are expected to continuously rise over the next 10 years by as much as 39 percent to reach 3.9 million tons by 2025/26 (Lee et.al., 2016). The Indian government has also been promoting the use of soybean products as a healthier alternative to traditional oils and proteins. The increasing demand for soybean products has encouraged the growth of the soybean processing sector in India, and the industry is expected to continue its growth trajectory in the coming years.

Opportunities for Growth in the Soy Processing Sector

Despite the challenges faced by the soybean processing sector in India, there are several opportunities for growth and development in the sector. Some of the major opportunities are:

Exports: India has a huge potential for exporting soybean products, particularly to countries in the Asia-Pacific region. The sector can leverage this opportunity by improving the quality of its products and increasing the production of value-added products. India was the 43rd-largest soy oil exporter in the world in 2020 with exports of $16.8M. The main destination of soybean oil exports from India are Bhutan, Canada, the United States, Mauritania and Australia (Observatory of Economic Complexity, 2022).

Government Support: The government has been actively promoting the soybean processing sector in India through various initiatives and policies. The sector can take advantage of this support to invest in research and development, increase the production of value-added products, and improve the competitiveness of the sector. In 2022-23 the government has increased the Minimum Support Prices (MSP) for soybean from 3950 to 4300 to ensure remunerative prices to the soybean growers for their produce (PIB, 2022).

Research and Development: There is a huge potential for growth in the soybean processing sector in India through research and development. The sector can invest in research to improve the quality of its products, increase the productivity of soybean crops, and develop new value-added products.

Increasing Awareness of Health and Nutrition**:** Soybean comprises, approximately 35% protein, 31% carbohydrate, 17% fats, 5% mineral, and 12% moisture (Messina and Lane, 2007). Soybean protein contains an acceptable amount of essential amino acid i.e. histidine, isoleucine, leucine, lysine, phenylalanine, tyrosine, threonine, tryptophan, and valine Soybean has been reported to impart several health benefits such as lowering of plasma cholesterol (Anthony et al., 1996), prevention of cancer (Kennedy, 1998), improvement in bone mineral density (Kreijkamp-Kaspers et al., 2004) and provide protection against bowel and kidney disease (Friedman and Brandon, 2001). These health benefits are caused by the presence of isoflavone, saponins, proteins and peptides in soybean (Friedman and Brandon, 2001; Michelfelder, 2009; Xiao, 2008). The growing awareness of health and nutrition among the people is driving the demand for soybean products in India. The sector can take advantage of this trend by investing in marketing and promoting the health benefits of soybean products.

Increasing Demand for Soybean Products: The demand for soybean products such as soy milk, tofu, and other vegetarian meat alternatives in India is increasing due to the growing awareness of health and nutrition among the people. The soy protein industry of India currently shares around 12% of the Asia-Pacific soy protein market share. Though the percentage share of soy protein in India is relatively low compared to China, it is projected that by the next decade, the gap will be reduced. The market revenue of soy protein in the country is projected to grow at a CAGR of 8% from 2022 to 2027 to capture a value of $478.7 million by 2027. The application of soy protein across the food industry shares the highest market share. (Research and Markets Forecast, 2022). The increasing demand for soybean products is expected to continue in the coming years, providing a huge potential for growth in the sector. The sector can leverage this opportunity by investing in research and development, increasing the production of value-added products, and improving the quality of its products.

Growing Livestock Industry: The livestock industry in India is growing rapidly, leading to an increased demand for animal feed, particularly soy-based feed. This presents an opportunity for growth in the soybean processing sector as soy meal is an important ingredient in animal feed.

Increasing Use of Soybean Oil in Industrial Applications: The increasing use of soybean oil in industrial applications, such as biodiesel, is providing opportunities for growth in the sector. In industry, soybean is used in the manufacture of edible lard, margarine, vegetable ghee, milk, and pastries, as well as the manufacture of paints, varnishes, adhesives, etc. (Narayan, 2017). The sector can leverage this opportunity by investing in the production of high-quality soybean oil for industrial purposes

Challenges Faced by the Soybean Processing Sector in India

Despite the growth of the soybean processing sector in India, the industry still faces several challenges that need to be addressed. Some of the major challenges faced by the sector are:

Lack of Processing Facilities: One of the major challenges faced by the soybean processing sector in India is the lack of processing facilities. Many farmers still sell their soybean crops directly to the market, which results in low value addition and low profitability. The government and private sector need to invest in building more processing facilities to increase the value addition of the soybean crop.

Low Productivity: The productivity of soybean crops in India is relatively low compared to other countries. This is due to a lack of investment in research and development, which results in poor quality seeds and poor agronomic practices. Improving the productivity of soybean crops will help the sector to meet the growing demand for soybean products in the country. India relies heavily on soybean imports, which makes it vulnerable to price fluctuations in the global market.

Quality Control Issues: Quality control is a major issue in the soybean processing sector in India. The lack of standardization in the sector results in products of varying quality, which affects the reputation of the industry and the country. The government needs to implement quality control measures to ensure that the products produced by the sector meet international standards.

High Cost of Inputs: The cost of inputs such as seeds, fertilizers, and labour is high in India, which affects the profitability of the soybean processing sector. The government and private sector need to take measures to reduce the cost of inputs and improve the competitiveness of the sector.

Lack of Technological Advancements: The soybean processing sector in India lacks the latest technology, which hinders the industry's ability to process soybeans effectively and efficiently.

Competition from International Players: The soybean processing sector in India faces stiff competition from international players, who have an advantage in terms of technology and economies of scale.

Lack of Government Support: The soybean processing sector in India lacks adequate support from the government in terms of subsidies and incentives, which affects its competitiveness and growth potential.

Environmental Concerns: The soybean processing sector in India faces challenges in terms of environmental concerns, such as pollution and deforestation, which need to be addressed to ensure sustainable growth.

Challenges in Distribution and Marketing: The soybean processing sector in India faces challenges in the distribution and marketing of its products, which affects its ability to reach its target customers.

Government Policies and Regulations Affecting the Soybean Processing Sector in India

The soybean processing sector in India is impacted by various government policies and regulations. Some of the key ones are:

Import-Export Policies: The Indian government regulates the import and export of soybean and its products through tariffs, duties, and quotas.

Agricultural and Food Processing Policies: The government provides support to the agricultural sector through subsidies, tax benefits, and other incentives to promote the processing of soybeans in India.

Food Safety and Standards Regulations: The Food Safety and Standards Authority of India (FSSAI) sets standards for the quality, safety, and labelling of soybean products, which processors must adhere to.

Environmental Regulations: The processing of soybeans generates waste and pollution, and the government regulates this through environmental laws and regulations to protect the environment.

Labour Laws: The government enforces laws related to the working conditions and wages of employees in the soybean processing sector, to ensure their welfare. These policies and regulations affect the operations and growth of the soybean processing sector in India, and companies must comply with them to remain competitive and sustainable.

Import and Export of Soybean Products in India

India is one of the largest importers of soybean products in the world. The country imports mainly soybean meal, which is used as a protein source in animal feed. The main sources of soybean meal imports are the United States, Brazil, Argentina, and Ukraine. In 2019-2020, India imported around 10.5 million tons of soybean meal, valued at $4.9 billion. On the export front, India mainly exports soybean oil, which is used for cooking and industrial purposes. The major destinations for soybean oil exports are Vietnam, Indonesia, the United States, and Malaysia. In 2019-2020, India exported around 2.5 million tons of soybean oil, valued at $2.4 billion. India's import and export of soybean products are significant and play an important role in the country's economy. The soybean industry provides employment opportunities and contributes to the growth of the agricultural sector. India was the 43rd-largest soy oil exporter in the world in 2020 with exports of $16.8M (Observatory of Economic Complexity, 2022).

Technological Advancements in the Soybean Processing Sector in India

Automated Processing: The use of advanced technology in soybean processing has led to the automation of various processes, including cleaning, dehulling, and oil extraction. This has increased efficiency and reduced the manual labour required for these processes.

Improved Extraction Methods: The introduction of improved extraction methods, such as the solvent extraction process, has significantly increased the yield of oil from soybeans. The solvent extraction process is faster and more efficient than traditional methods, making it a preferred method in soybean processing.

Use of Advanced Equipment: The use of advanced equipment in soybean processing, such as centrifuges and dehulling machines, has improved the quality of the final product. These machines are designed to remove impurities, improve the efficiency of the process, and increase the yield of oil from soybeans.

Increased Capacity: The adoption of technology has increased the capacity of soybean processing units in India. This has enabled companies to process more soybeans in less time, reducing the time taken to produce the final product.

Better Quality Control: The use of technology in soybean processing has also improved the quality control process. Advanced equipment and software systems monitor the entire processing process, ensuring that the final product meets the required quality standards.

Energy Efficiency: The use of technology has also made the soybean processing sector in India more energy-efficient. Advanced equipment and processes consume less energy, reducing the carbon footprint of the sector and making it more environmentally friendly.

Environmental Impact of Soybean Processing in India

The processing of soybeans in India has a significant impact on the environment. Some of the main environmental impacts are:

Deforestation: The increased demand for soybeans has led to deforestation of large areas of land to make way for soybean cultivation. This has resulted in the loss of valuable habitats for wildlife and has contributed to soil degradation.

Water Pollution: The processing of soybeans requires large amounts of water, which can lead to water pollution if the water is not properly treated before it is released back into the environment.

Greenhouse Gas Emissions: The production and transportation of soybeans generate significant amounts of greenhouse gases, including carbon dioxide, methane, and nitrous oxide.

Pesticide and Fertilizer Use: Soybean cultivation often involves the use of pesticides and fertilizers, which can contaminate the soil and groundwater and harm wildlife.

Soil Erosion: The removal of vegetation for soybean cultivation can lead to soil erosion and loss of fertile land. The processing of soybeans in India has negative impacts on the environment, including deforestation, water pollution, greenhouse gas emissions, pesticide and fertilizer use, and soil erosion. To minimize these impacts, it is important for the industry to adopt sustainable practices, such as using recycled water, reducing pesticide and fertilizer use, and conserving habitats for wildlife.

Employment Prospects of the Soy Processing Sector

The soybean processing industry in India holds good employment prospects. According to a report by the Indian Ministry of Agriculture and Farmers Welfare, the soybean processing industry in India employs around 1.5 million people, with the majority of them being skilled and unskilled workers. The soybean processing industry in India has been growing steadily in recent years. India is the world's fourth-largest producer of soybeans and the demand for processed soy products has been increasing due to the growth of the food and feed industries.

The soybean products processing sector, with a current overall market of 18.35 MMT engaged 0.06 Lakh employees in FY 2020. Currently, there are 165 registered soy processing units, with 4.8% past growth rate (i.e., from FY 2015-2020). The overall export value of soy-processed products was reported worth Rs. 3,681 Crores. The soy processing sector is expected to witness its growth during 2021-30 with 34 MMT as the overall market, 6.4% growth, and 0.11 Lakh employees in processing units in FY 2030. Thus, the net expected skilled human resource requirement in this sub-sector during 2021-30 would be around 0.05 Lakh. (FICSI & MoFPI, 2022). This has led to an increase in the number of new companies entering the market and creating new job opportunities. The soybean processing industry in India holds good employment prospects, particularly in areas such as production, marketing, and research and development. With the growing demand for processed soy products and the government's support, the industry is expected to continue to expand and create new job opportunities.

Future Prospects for the Soybean Processing Industry in India

The soybean processing industry in India is expected to experience strong growth in the coming years, due to increasing demand for soy-based products such as tofu, soy milk, and livestock feed. The Indian government has also taken steps to promote the growth of the industry, such as increasing import duties on soybean oil and providing subsidies for soybean farmers. Additionally, the growing middle class in India, combined with increasing health consciousness, is expected to drive demand for soy-based products. However, the industry may face challenges such as competition from other oilseeds, fluctuating commodity prices, and limited infrastructure for processing and storage. The government has launched various schemes to promote the cultivation of soybean, such as the National Food Security Mission and the Rashtriya Krishi Vikas Yojana. These initiatives aim to increase the area under soybean cultivation and to improve the yield and quality of soybeans produced in India.

CONCLUSION

The soybean processing industry in India has come a long way in the last few decades. The country is one of the largest producers of soybeans in the world, and the industry is expanding rapidly. With a growing demand for soy products, the industry has a significant potential for growth in the future. The government is also playing a crucial role in promoting the industry by providing incentives and subsidies for farmers, processors, and exporters. The increasing demand for soy-based food and non-food products, coupled with the growing awareness about the health benefits of soy, is driving the growth of the industry. The industry has the potential to create new job opportunities and contribute to the growth of the Indian economy. The soybean processing sector in India is growing rapidly, with ample opportunities for investment and business. The sector faces several challenges, including the lack of processing facilities, low productivity, quality control issues, and high cost of inputs. However, there are several opportunities for growth, including exports, government support, research and development, increasing awareness of health and nutrition, and increasing use of soybean oil in industrial applications. The sector can leverage these opportunities to overcome the challenges and grow further in the coming years.

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Breaking Ground: Nano-Enabled Solutions for Amplifying Soybean Crop Yield and Seed Quality

RAME GOWDA, ROOPASHREE B, UMA RANI K AND SOWMYA KJ
Seed Technology Research Centre, AICRP on Seed (Crops), University of Agricultural Sciences, GKVK, Bangalore 560 065, India
E-mail: roopagowda152@gmail.com
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ABSTRACT

Rapid climate change and a rising worldwide population have increased the demand for soybean. Advances in nanotechnology have recently revolutionized agricultural practices on a worldwide scale in order to increase crop output. This technique not only has an influence on the agricultural market but also opens up new avenues for agricultural-related industrial use of nanomaterials. As a result, during the last two decades, soybean has attracted attention to the use of nanotechnology with significant advances in seed science research. In this review, information on the use of nanoparticles in increasing soybean seed development, yield, storage life, and seed health are gathered and summarized.

Keywords: Soybean, nanoparticles, seed yield, seed quality enhancement, seed health.

Soybean (*Glycine max* L.) is one of the most important world crops and is grown for its oil and protein. In 2019, the FAO classified soybean as the fifth largest crop worldwide in terms of agricultural productivity (Yusefi-Tanha *et al*., 2020). Globally, soybean covers 136.82 million hectares with a production of 353.47 million tonnes (Soybean Outlook, 2022). In India, soybean is grown over an area of about 12.81 million ha with a production of 12.90 million tonnes and productivity of 1007 kg/ha. But the total area coverage under soybean in Karnataka is 0.31 million ha with a production of 0.38 million tonnes and a productivity of 1212 kg/ha (Agricultural Statistics at a Glance, 2021) which is slightly better than all India's average during 2020-21. Soybean is a precursor for biodiesel and biomaterials, and it is now the leading producer of oilseeds. Despite the high yielding potential and benefits, soybean agriculture yields less per unit area in India. One of the major issues restricting soybean output is a lack of quality seeds with poor germination, vigour, and shelf life. Soybeans utilize 50% of their weight in moisture to germinate, but maize absorbs just 30%, hence they must be planted in soils with appropriate moisture to enable optimal germination and seedling establishment. To maintain high soybean yields, researchers have focused on the availability of several micronutrients in the soil using nanofertilizers, which have a high surface area to volume ratio and can be easily absorbed by plants (Elemike *et al*., 2019; Ajilogba *et al*., 2021). The nanoparticles make their way into irrigation water and soil, where they interact with plants more effectively, resulting in increased agricultural output. Nanoparticles for seed treatment are gaining popularity because they are more effective than traditional agrochemicals, making them both economically and environmentally feasible. Nanotechnology has the potential to greatly contribute to the long-term development of nanoscale agrochemicals for seed treatment and to improve the efficiency of agricultural inputs. Nanoparticles have been shown to boost seed germination, biomass, and seed yields. The nanoparticles also improved the seed's tolerance to a variety of biotic and abiotic stress. The use of nanoparticles in the soybean crop has been extensively researched in order to improve germination, vigour, resistance to biotic and abiotic stresses, and crop yield. Recent studies have discovered that seeds treated with nanomaterials can activate several genes during germination (Wang *et al*., 2022).

Nanoparticles in improving seed quality, seed growth and yield

Over the last ten years, a great deal of research has been done on the bioaccumulation and biotransformation of ZnO and CeO_2 NPs in soybean cultivated under hydroponic, greenhouse, or nanoparticles (NPs) influenced soil conditions (Priester *et al*., 2017; Yoon *et al*., 2014). For the first time, López-Moreno *et al*. (2010) demonstrated a material-dependent effect on soybean plants by illustrating that cubical CeO_2 NPs (7 nm) were stored in the roots of the plants grown hydroponically while hexagonal ZnO NPs (8 nm) could only be detected in the roots as the Zn (II) ionic form.

A few years later, the first study of a soybean grown to maturity in NP-impacted soil revealed that plant growth (root and shoot length, number of leaves) was negatively impacted even at low CeO_2 NPs (8 nm) concentrations (0.1 g/kg soil), but slightly stimulated by ZnO NPs (8 nm) at comparable soil concentrations (0.05–0.1 g/kg) (Priester *et al*., 2012). CeO_2 accumulation, as determined by energy dispersive X-ray spectroscopy (EDX) and dark-field scanning transmission electron microscopy (TEM), occurred primarily in the roots and nodules (with very little aboveground translocation) at rates comparable to those from the prior hydroponic study (Lopez-Moreno *et al*., 2010). In a subsequent investigation, Hernandez-Viezcas *et al*. (2013) demonstrated using X-ray absorption spectroscopy that ZnO (10 nm) NPs were not present; instead, different Zn complexes (such as Zn citrate or nitrate) were found within the stem, leaves, and beans, but none as NPs. In contrast, CeO_2 (8 nm) NPs remained primarily as particles in the root and nodules. This study also showed that some Zn^{2+} ions and CeO_2 nanoparticles entered the soybean plant's reproductive and edible parts, indicating that CeO_2 NPs may be able to enter the food chain and the subsequent plant reproduction. Further, Peralta-Videa *et al*. (2014) demonstrated that CeO_2 (8 nm) and ZnO (10 nm) NPs had various impacts on the accumulation of nutrients in soybean plants growing in NP-impacted agricultural soil, hence changing the nutritional value of the plants. CeO_2 exposure (0.1–1g/kg) mostly affected the absorption of the substances necessary for nitrogen metabolism and photosynthesis: Ca, Mg, K, and Na levels in pods dramatically dropped, although P and Cu levels improved. Most of the accumulation of critical elements was altered by ZnO exposure (0.05–0.5g/kg), which increased Zn, Mn, and Cu in pods and Mo in nodules while lowering Fe accumulation in leaves. All of the examined soybean organs, including the pods, had considerable amounts of Zn, much like in the prior research (Hernandez-Viezcase *et al*., 2013). While the majority of the aforementioned studies assessed the impact on plant growth, a subsequent study from the same groups sought to assess the impact on soybean metabolism in terms of chlorophyll production, seed protein content, ROS, lipid peroxidation, and genotoxicity of CeO_2 (8 nm) and ZnO NPs (10 nm) (Priester *et al*., 2017). Although exposure to both NPs exhibited undesirable consequences, CeO_2 NP exposure caused the most obvious harm. Increased ROS and lipid peroxidation, as well as reduced total chlorophyll concentrations, were more significant at medium (0.5g/kg soil) and high (1.0g/kg soil) levels. These impacts were closely associated with leaf damage, decreased pod and stem formation, and a reduced capacity of root nodules to fix nitrogen. ZnO had no effect on plant growth, yield, or N_2 fixation capability, although a dose-dependent mild oxidative stress was seen along with slightly decreased chlorophyll concentrations (attributed to Zn complexes). While ROS levels were similar to the control, one plant treated with the highest measured dose of ZnO (0.5g/kg soil) showed some signs of genotoxicity, which was mostly due to ionic Zn oxidative phytotoxicity.

The results of a different study with plants grown in a standard soil microcosm (57–65 days) contradict the mild effects of ZnO on soybeans. This study found that ZnO NPs (50 nm) at 0.05 and 0.5g/kg soil significantly affected the development of the plant's reproductive system. At the highest concentration (0.5g/kg), roots were smaller and no seeds were produced. The ZnO NPs in this investigation were an order of magnitude bigger than those in the other experiments, and as the NPs' characteristics are strongly size-dependent (Jiang *et al*., 2008; Albanese *et al*., 2012), their size may have had an impact on their biological effects. Smaller ZnO particles have been found to dissolve in soil and enter plant cells as Zn^{2+} cations, but bigger particles have been demonstrated to enter cell compartments as NPs and have physiologically distinct effects (Xia *et al.*, 2008). Smaller sizes should have faster dissolving rates due to an increase in surface-specific area (Ma *et al.*, 2013).

In another investigation, 0.25-1 mg/mL exposure to CeO_2NPs (25 nm) had no effect on soybean seed germination or early development root length (Andersen *et al.*, 2016). Dan *et al.* (2016) discovered dissolved Ce for the first time in plant seedling shoots exposed to CeO_2NPs in hydroponically grown plants. The soil moisture content was demonstrated to alter the impact on soybean, with beneficial impacts above 70% humidity (Cao *et al.*, 2017; Cao *et al.*, 2018). In terms of interactions with other co-contaminants, it was discovered that CeO_2 and Cd interact considerably, altering each other's accumulation and, as a result, their biological impacts (Rossi *et al.*, 2017; Rossi *et al.*, 2018). The effects were concentration-dependent, as predicted (Servin *et al.*, 2017), and soil sterilization was found to have a major impact on the effect of NPs, most likely owing to changes in soil microbiota (Stowers *et al.*, 2018). The majority of research concluded that the breakdown of ZnO to Zn ions is the most important factor in the impacts of NPs on soybean plants. Dissolution was particle size-dependent, as predicted, with smaller NPs more prone to solubilization. Proteins and organic compounds can also speed up ZnO dissolution *via* ligand-enhanced dissolution (Xia *et al.*, 2008). ZnO was distributed in all soybean plant organs in a concentration-dependent way in terms of bioaccumulation in soybean plants. The presence of Zn in exposed plants' edible sections raises concerns about potential transgenerational impacts, trophic transmission, and bio-magnification (Gardea-Torresdey *et al.*, 2014). In contrast to ZnO, CeO_2 was found to be mostly prevalent in the roots and nodules of the soybean plant and was not susceptible to biotransformation. Because CeO_2 NPs are durable and insoluble in biological systems, they may be discovered as NPs in soybean roots. CeO_2 was shown to have both good and negative effects in soybean plants, serving as an antioxidant or a ROS generator.

Fe_2O_3 NPs have been shown to have a primarily positive effect on soybean growth in terms of biomass increase (Sheykhbaglou *et al.*, 2010), concentration-dependent protein, lipid, fatty acid, and mineral concentrations (Sheykhbaglou *et al.*, 2018), root elongation and shoot weight (Alidoust and Isoda, 2013), chlorophyll content (Ghafariyan *et al.*, 2013). However, in the most recent study, higher lignin concentration in roots hampered plant development (Cunha Lopes *et al.*, 2018). Foliar spraying (Sheykhbaglou *et al.*, 2010; Sheykhbaglou *et al.*, 2018; Alidoust and Isoda, 2013), medium (Ghafariyan *et al.*, 2013; Cunha Lopes *et al.*, 2018), and soil amendment (Alidoust and Isoda, 2013) were used to apply Fe_2O_3 NPs.

Alidoust and Isoda (2013) confirmed that foliar treatment of Fe_2O_3 (6 nm;

0.05-2 g/L) outperformed soil amendment in terms of photosynthetic efficiency. Additionally, improved stem and root development, as well as total yield, were reported, with the benefits being more dramatic than with Fe in the ionic form (salts). According to Burke *et al*. (2015), in contrast to positively charged NPs, negatively charged NPs raised leaf P concentration, promoted the transfer of Fe to leaf tissues, and inhibited rhizobial root colonization. Liu *et al*. (2019) showed that the negatively charged Fe_3O_4 NPs may be more biocompatible and absorbable by plants. To determine if Cr_2O_3 NPs can be absorbed and translocated by plants, as well as whether they may have an impact on the synthesis of chloroplasts and chlorophyll, research using Cr_2O_3 NPs (30–50 nm; 0.01–0.5 g/L) and soybean was conducted (Li *et al*., 2018). By reducing the activity of the photosynthetic enzymes (malate dehydrogenase and Ribulose-1,5-bisphosphate carboxylase / oxygenase—Rubisco), altering the substructure of chloroplasts, and decreasing the formation and activity of chlorophyll, the authors have demonstrated that Cr_2O_3 NPs caused root swelling and decreased plant growth, resulting in irreversible damage to the soybean plants.

CuO NPs (50 nm) had a comparable effect in terms of increased lignin content in the root and decreased root development in soybean as Fe_2O_3 (Cunha Lopes *et al*., 2018; Nair *et al*., 2014). The elevated activity levels of both cationic and anionic peroxidases explained the impact (Lin *et al*., 2005). The interaction of Ag NPs with soybean was found to have no deleterious effect on germination and growth (Guilger *et al*., 2017). Ag reduced flooding stress by switching from fermentation to normal cellular activities (Mustafa *et al*., 2015) and decreased the detrimental impacts of dichloro-diphenyl-dichloro- ethylene (DDE; a co-contaminant) by slowing its accumulation rate (De La Torre-Roche *et al*., 2013). However, recent research by Galazzi *et al*. (2019) using transgenic and non-transgenic soybean plants had shown that biomass production was decreased in the presence of Ag NPs, indicating that the soybean plants were under oxidative stress. Engineered nanometals have been utilized as micronutrients or stimulants to increase agricultural output and quality. According to Pham *et al*. (2018), the treatment of soybean seeds with Fe, ZnO, Cu, and Co NPs at various concentrations increased the rate of germination and the development of primary roots and leaves. The root growth acceleration effect of ZnO NP seemed to be the greatest. From seedlings treated with ZnO and Co NPs, leaves grew noticeably more quickly. The development of primary leaves was not influenced by Fe or Cu NPs. The root mitotic index, which rose from 26 to 34% compared to the control, also showed the favorable impact of metal NPs.

Zn can be transferred from the coatings to the seedlings (Montanha *et al*., 2020). After planting, the majority of the Zn on the seed coat was transported to the soil, and less than 13 wt% was absorbed by the 14-day-old seedling after sowing, as determined by X-ray spectroscopy. Before germination, Zn did not cross the seed coat or undergo biotransformation. Either during the imbibition phase or during seedling development, this trace amount of Zn was absorbed. To promote soybean germination and seedling growth, zinc oxide-based therapies stand as a feasible alternative to Zn supplementation.

Different concentrations of TiO_2 and ZnO nanoparticles (10, 100, and 1000 mg/L) on soybean plant *in vitro* were examined for potential effects on the environment and human health (Leopold *et al*., 2022). The stomatal conductance to water vapour decreased and the plant stress levels were greater in the soybean plant as shown by the net CO_2 assimilation rate. Additionally, ZnO nanoparticle treatments had a negative impact on plant development, and TEM investigation showed that the chloroplasts had changed ultra structurally and that the cell walls of the leaves had ruptured. Results from ICP-OES and TEM analysis revealed that the nanoparticles were metabolized and internalized in the tissues of the soybean plant in ionic forms. Most likely, this behavior serves as the primary catalyst for nanoparticle toxicity. Yusefi Tanha *et al*. (2022) investigated the effects of ZnONP particle size (38, 59, and > 500 nm) and concentration (0-500 mg/kg) on Zn bioaccumulation in multiple tissues in soil-grown soybean for 120 days, including changes in RSA (root biomass, length, area, volume, and density) and soil characteristics (pH and electrical conductance [EC]). While the concentration-response curves for Zn distribution in several tissues were linear, they were judged nonlinear for root structures with a range of sized ZnONPs and Zn^{2+} ions. Up to 200 mg/kg, ZnONPs, and Zn^{2+} ions had positive effects on root growth and development, but larger doses of both substances caused hazardous reactions. In comparison to the bigger-sized ZnONPs (59 and > 500 nm) and Zn^{2+} ions, soybean responses to the lowest-size ZnONPs were generally greater for all parameters assessed. The order of the tissues' Zn bioaccumulation was: root > seed > leaf > stem.

Yusefi-Tanha *et al*., 2023, investigated the particle size (S [small]= 38 nm, M [medium]= 59 nm, and L [large]=> 500 nm), and concentration-dependent effects of nZnO on photosynthetic pigments, pod development, potassium and phosphorus accumulation in soybean seed, protein, and oil yields. In comparison to nZnO-M, nZnO-L, and Zn2+ ions treatments up to 200 mg/kg, soybean exhibited substantial stimulatory responses to nZnO-S for the majority of the parameters examined, indicating the potential for small-size nZnO to enhance seed quality and production. Toxic effects were seen with all Zn compounds at 500 mg/kg, but not for carotenoids or seed development. Additionally, a

TEM study of seed ultrastructure revealed that seed oil bodies and protein storage vacuoles may change at a hazardous dose of nZnO-S (500 mg/kg). The best dose of 200 mg/kg of the lowest size nZnO-S (38 nm) considerably increases soybean seed production, nutritional quality, and oil and protein yield. According to Ngo *et al*. (2014), soybean seeds treated with an extra-low nanocrystalline dosage (no more than 300 mg of Cu, Co, and Fe per hectare) can consistently alter the biological indicators of plant growth and development. Furthermore, under drought-induced circumstances, soybean plants treated with iron, copper, cobalt, and zinc oxide nanoparticles exhibited considerably higher relative water content, a higher drought tolerance index, and a faster rate of biomass loss. Three drought-responsive genes showed increased expression in the leaves of all NP-treated plants, and *GmERD1* (Early Responsive to Dehydration 1) expression was induced in both roots and shoots, indicating that NP application can increase the drought tolerance of soybean plants by inducing drought-associated gene expression (Linh *et al*., 2020).

Biogenic metallic nanoparticles are showing promise as a nano-fertilizer and a phytopathogen control option. AgNP treatment causes oxidative stress and a loss in root dry weight in soybean plants and seedlings, but it also causes adaptive responses including a decrease in stomatal conductance without affecting photosynthesis and an improvement in intrinsic water usage efficiency. A defence mechanism through root lignification is shown by the seedlings exposed to FeNPs showing an increase in oxygen peroxide levels in the leaves without accompanying lipid peroxidation and an increase in *POD2* and *POD7* gene expression (Guilger-Casagrande *et al*., 2022).

The nanoscale Co- and MoO_3-microelement containing formulations may be employed successfully as a pre-sowing seed treatment for better soybean plant growth and germination as well as higher amylase and lipase activities Chau *et al*. (2019).

The soybean plants exposed to metal-based nanoparticles exhibited changes in DNA methylation, a key epigenetic mechanism for regulating gene expression, as well as an uptick in the expression of representative germination-related genes, which code for enzymes involved in ethylene biosynthesis and the mobilization of seed reserves. By controlling DNA methylation and associated gene expression, they promote soybean seed germination and seedling development (Linh *et al*., 2021).

An appealing method to prevent the molecules from degrading quickly, permitting their usage in agriculture, and increasing their range is the nano-encapsulation of nitric oxide (NO) donors. By increasing the bioavailability of nitric oxide in the roots, causing a more significant induction of the antioxidant activity, the attenuation of oxidative damage, and a greater capacity to mitigate the root nutritional imbalance brought on by Cu stress, the exogenous application of the nano-encapsulated S-nitroso-mercapto-succinic acid improved the growth and promoted the maintenance of the photosynthetic activity in Cu-stressed plants (Gomes *et al*., 2022). The use of nanoscale hydroxyapatite (nHA) as a P fertilizer might increase crop yields and biomass output. When compared to soybeans treated with [$Ca(H_2PO_4)_2$] fertilizer, the nHA nanoparticle treatment enhanced growth rate, seed production, and biomass. As a novel kind of P fertilizer, apatite nanoparticles have the potential to increase crop productivity while lowering the danger of water eutrophication (Liu and Lal, 2014). AgNPs are thought to play a crucial role in the metabolic switch from fermentative pathways to normal cellular processes and the production of very few cytotoxic by-products in soybeans treated with AgNPs during flooding stress. AgNPs mainly influence the levels of proteins that are involved in stress, signaling, and cell metabolism (Mustafa *et al*., 2015a). Under flooding circumstances, Al_2O_3 nanoparticles reduce cell death in roots, including the hypocotyl, and mediate the transition from anaerobic to aerobic energy metabolism. The major effects of Al_2O_3 nanoparticles on proteins involved in energy metabolism and cell wall production. Under flooding stress, proteins associated with glycolysis are decreased by Al_2O_3 nanoparticles. These responses are considered to be key factors for improving the growth performance of soybeans under flooding stress (Mustafa *et al*., 2015b). Depending on the nature and place of NPs' production, different plants responded differently to them.

Bio-synthesized (BS) silver NPs promote soybean development by modulating proteins involved in protein breakdown and ATP content, which are negatively affected by chemically synthesized (CS) silver NPs (Mustafa *et al*., 2020). Under drought conditions, nano zinc oxide increases the soybean germination percentage and changes the quality of soybean performance in field conditions (Sedghi *et al*., 2013). Lu *et al*. (2002) reported that a mixture of nano-SiO_2 and nano TiO_2 might boost the nitrate reductase in soybeans, improve their capacity to absorb and use water and fertilizer, activate their antioxidant system, and ostensibly hasten their germination and growth. Even nano-anatase has been shown to improve soybean germination and growth (Lu *et al*., 2002). Further, in the research of Adhikari *et al*. (2012), seed treatment with copper (Cu) oxide nanoparticles had no effect on the germination of soybeans, although it did shorten the roots. However, the treatment of soybean seeds with iron, cobalt, and copper nanocrystalline powder enhanced the germination percentage of the seeds (Ngo *et al*., 2014). Single-walled carbon nanotubes (SWCNTs) have been shown to improve the germination of tobacco, maize, soybean, switchgrass, tomato, rice, and barley (Milewska-Hendel *et al*., 2016). Based on Lopez-Moreno *et al.* (2010), ZnONPs had no discernible impact on the germination of soybean seeds, although they did promote root length at lower concentrations (500 ppm) and inhibit it at higher concentrations (4000 ppm and 5000 ppm).

Nanoparticles maintain seed shelf life during storage

Farmers in underdeveloped nations frequently unintentionally utilize subpar seeds purchased from neighbourhood markets (Sperling *et al.*, 2020). Low germination and production can be caused by poor seeds (De Vitis *et al.*, 2020). Although seed quality cannot be increased while being stored, it can be maintained until the appropriate time for planting by altering the storage environment (Brito *et al.*, 2020). Farmers always want high-quality seed since it may enhance crop yields by up to 30%. Because of their high fat and protein content, soybean farming is significantly hampered by the seeds' rapid deterioration when stored. To increase the shelf life of seeds, coating technology with different coating formulae is required. In comparison to untreated control, the application of chitosan-wax nanoparticles (CSNPs) have been considerably reduces the rise in water content and the decrease in protein and fat levels in soybean. In addition, the coating reduces the concentration of MDA, ethylene content, and respiration rate during storage (Arif *et al.*, 2023). An effective substitute for seed coating that will enhance seed performance while being stored is nano-chitosan.

The homogeneity of seed coverings can be enhanced by the amphiphilic polymers' nanoscale and broad-spectrum solubility (they include both hydrophilic and hydrophobic blocks). It can be used to prevent aging in soybean seeds. With little environmental contamination, formulations used as seed treatments may offer increased and long-lasting effectiveness (Adak *et al.*, 2016). Similar to this, seeds dry dressed with FeNPs @ 500 ppm nanoparticles lower the rate of seed degradation in storage by preserving better seed germination and seedling vigour (Sandeep *et al.*, 2019).

Nanoparticles as seed health maintainers

Polymeric nanoparticles (PNPs) have been proposed as suitable delivery systems for a variety of agricultural applications, including herbicide delivery (Tong *et al.*, 2017), insecticide delivery (Gabriel Paulraj *et al.*, 2017; Lichtenberg *et al.*, 2020), fungicide delivery (Sandhya *et al.*, 2017; Yang *et al.*, 2014), and plant growth regulator delivery (Pereira *et al.*, 2017). Antifungal seed treatments for soybeans can include zein nanoparticles (ZNPs) and lignin-graft-poly (lactic-co-glycolic) acid nanoparticles (LNPs). Seed treatment with ZNP and LNP was shown to have no effect on germination and may be useful for active chemical delivery methods in seed treatment. When employed as a seed treatment, azoxystrobin-loaded (technical grade) LNPs provided practically total antifungal protection (100%) for soybean plants against *R. solani* (Kacso *et al*., 2022). The findings indicate that ZNPs and LNPs are safe and efficient active chemical delivery methods for seed treatments. Previous research has also revealed that positively charged ZNPs have an affinity for hydroponic soybean roots (Ristroph *et al*., 2017). Negatively charged core-shell LNPs synthesized without the use of a surfactant (Astete *et al*., 2020) were shown to be safe for soybean plants in hydroponic systems at doses of up to 2 mg/mL (Salinas *et al*., 2021). Furthermore, leaf-feeding assays and damage indices of soybean plants showed that lignin or zein nano seed treatment does not enhance sensitivity to herbivore attack (Bonser *et al*., 2023). Thus the NPs have also been very much involved in plant protection against biotic stress and resulted in better crop growth and yield and quality.

Nanoparticles in stabilizing soil microbiome

CeO_2 (nanorods; 67×8 nm^2) and ZnO (spherical NPs; 24 nm) showed an effect on soil bacterial communities where, ZnO significantly impacted bacterial communities in both unplanted and planted soils, increasing *Rhizobium* and *Sphingomonas* while decreasing *Ensifer, Rhodospirillaceae, Clostridium*, and *Azotobacter*. The presence of soybean plants showed varying impacts on ZnO-affected soil bacterial populations (Ge *et al*., 2014). In planted soils, soybean reduced the impact of ZnO NPs on bacterial communities (50% fewer sensitive bacterial operational taxonomic units), most likely by immobilizing the NPs via root exudates or absorbing Zn ions via the roots, limiting the availability of Zn to soil bacteria. It is worth noting that ZnO has already been proven to have good antibacterial capabilities (Azam *et al*., 2012). CeO_2, on the other hand, had minimal influence on bacterial populations even at the lowest dose examined (0.1 g/kg). In this case, CeO_2 had a shifting effect on bacterial communities only in planted soils, which was attributed to the previously reported detrimental effect on plant roots, where plants exposed to this concentration had significantly shorter roots than CeO_2 NP-free controls (Priester *et al*., 2012). The parallels between the two experiments were also verified at higher concentrations, where the impacts of CeO_2 on the microbial communities and the plant itself were limited (Priester *et al*., 2012; Ge *et al*., 2014).

CONCLUSION

In order to augment the effectiveness of seeds, lessen the environmental contamination, enhance the sustainability of agricultural systems, and increase food security, nanotechnology can be safely used. Nanoparticle seed treatment for soybean improves the accuracy and efficacy of seed protection products by lowering the concentration of pesticides and fertilizers administered to the soil. For sustainable agriculture, nanocarriers with controlled distribution have a number of benefits over traditional chemical delivery methods, including biocompatibility, biosorption rate, cheap synthesis costs, thermo-plasticity, and simplicity of biodegradation. New generation seed treatment methods may be created by generating nanoscale materials such as nanocapsules, nanogels, nanofibers, nanoclay, and nanosuspensions. We can now articulate with certainty that employing nanoscale treatment for soybean is crucial for germination improvement and that they play a decisive role in sustainable crop production.

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Effect of Pre and Post Emergence Herbicides Alone and Mixtures on Soil Enzyme, Microflora and Yield of Soybean

UDITI DHAKAD, CK JADON, RK YADAV, DL YADAV, SL YADAV AND SN MEENA
College of Agriculture and Agriculture Research Station, Ummedganj, Kota Agriculture University, Kota, 324001, Rajasthan
E-mail: uditi1303@gmail.com
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ABSTRACT

Herbicide application for controlling weeds in soybean crop is an important part of plant protection in agriculture. In this study, among the various application of herbicides, maximum leaf area index, chlorophyll content, nodules/plant, dry weight/plant were recorded by fomesafen + fluazifop-p-butyl 220 g a.i./ha, propaquizafop + imazethapyr 125 g a.i./ha, and sodium acifluorfen + clodinafop-propargyl 245 g a.i./ha as compared to other applied herbicides. Pre-emergence application of pendimethalin + imazethapyr 960 g a.i./ha and pendimethalin 1.0 kg a.i./ha significantly reduced dehydrogenase enzyme activity compared to control. However, maximum phosphatase enzyme activity (30.17 µgpnp/g soil/hr) was observed under two hand weeding followed by fomesafen + fluazifop-p-butyl 220 g a.i./ha. All herbicidal weed control measures applied as pre-emergence and post-emergence did not influence total bacterial, fungi, actinomycetes populations. Herbicides application had not significant effect on the soil microbial population and soil enzymes.

Keyword: *Herbicides, Soil Enzyme, Soil microflora and yield*

Soybean [*Glycine max* (L) Merrill] has emerged as a potential protein as well as oilseed crop globaly. It has attracted the Indian farmers especially Rajasthan, Maharashtra and Madhya Pradesh due to its wider adaptability and high yield potential as compared to any other oilseed and pulse crop in the *kharif* season. In India, soybean occupied an area of 11.84 m ha with the production of 10.45 mt during 2020 (Anonymous, 2021). Rajasthan occupied an area of 11.29 lakh ha and production of 10.94 lakh tonnes having hovering productivity of 969 kg/ha (Anonymous, 2020-21). The productivity of soybean in the state of Rajasthan is deplorable low as compared to Madhya Pradesh (1231 kg/ha), Maharashtra (1132 kg/ha), India (1192 kg/ha) and the world (2491 kg/ha. World market and trade USDA, 2022). Soybean being a rainy season crop, suffers severely due to competitive stress of weeds. Weeds predominantly associated with soybean were *Echinochloa* *crusgalli, Echinochloa colona, Commelina benghalensis*, *Panicum dichotomiflorum, Polygonum spp*., *Aeschynomene indica* and *Digitaria sanguinalis,* however, *Eleusine aegyptium* and *Cyperus* spp. Weeds make a complete seizure of the land in the early stage of soybean crop growth. Yield reduction in soybean due to weeds may vary from 30-80 per cent (Gupta et al., 2006). Weed management in soybean had really been a challenging factor mainly in *kharif* season due to unpredictability of rains, entailing to non-workable conditions of soil in rainy days and timely non-availability of labour. Works on various herbicides have been done so far but for the dynamic evaluation of recent available new herbicides bioefficacy microbial activity and enzymesare very meager in the soybean growing regions particularly in the Rajasthan state which is a great concern to the safe cropping system and sustainability. Soil enzymes also play a vital role in maintaining the physical and chemical properties and thus, conserve soil ecology as well as soil health (Paul 2007). Kepler *et al*. (2020) found that glyphosate did not affect the overall microbial community composition in maize or soybean grown soil. Studying the effects of herbicides on the microorganisms and enzyme activity in vertisol soil is helpful to explicate the mechanisms of herbicides affecting both plant growth and soil environment.

MATERIAL AND METHODS

A field experiment was carried out in soybean [*Glycine max* (L.) Merrill] crop during *kharif*2021 at Agricultural Research Station, Ummedganj, Kota (Agriculture University, Kota) which is situated at $25^0 13'$ N latitude and $75^0 25'$ E longitude at an altitude of 258 m above mean sea level. This region comes under Agro-climatic zone V *i.e.* Humid South Eastern Plain of Rajasthan. The experiment was laid out in randomized block design (RBD) with sixteen treatments and three repetitions. Soybean crop was sown with the cultivar of JS 20-34 and seed was treated with carbendazim @ 1 g/kg. A uniform recommended fertilizer dose of nitrogen, phosphorus and potash (20:40:40 kg/ha) was drilled in furrow at a depth of 8-12 cm at the time of sowing. Nitrogen, phosphorus and potash were applied through urea, single super phosphate and muriate of potash, respectively. Sowing was done by tractor drawn seed drill kept in row space 30 cm apart a depth of 2-3 cm using a seed rate is 80 kg/ha. Weed management practices of hand weeding and herbicides application has been adopted as per experimental treatment in each earmarked plots as per schedule. Pre-emergence herbicides *i.e.* pendimethalin 1.0 kg a.i. /ha and pendimethalin + imazethapyr 960 g a.i./ha were applied just after sowing of soybean. Whereas, all the post emergence herbicides i.e. imazethapyr 100 g a.i./ha, fluthiacet-methyl 12.5 g a.i./ha, clodinafop propargyl 60 g a.i./ha, fomesafen 250 g a.i./ha, fluazifop-p-butyl 250 g a.i./ha, propaquizafop 50 g a.i./ha, propaquizafop + imazethapyr 93.75 g a.i./ha, propaquizafop + imazethapyr 125 g a.i./ha, sodium acifluorfen + clodinafop propargyl 183.7 g a.i./ha, sodium acifluorfen + clodinafop propargyl 245 g a.i./ha, fomesafen + fluazifop-p-butyl 165 g a.i./ha and fomesafen + fluazifop-p-butyl 220 g a.i./ha were applied with use of 0.1 per cent non-ionic surfactant at 16 DAS. All the herbicides were sprayed through knapsack sprayer using flat fan nozzle using 500 litre water/ha as per treatments.

Leaf area index (LAI)

The leaves of five randomly selected plants at 50 % flowering were detached after plant removal from the plot. These were categorized as small, medium, large and extra-large and counted. The representative leaf of each category was directly fed to the leaf area meter to work out the total leaf area of five plants used for calculating LAI by the formula given by Sestale *et al.*, 1971.

Leaf area index (LAI) =

$$\frac{\text{Leaf area per plant (cm}^2\text{)}}{\text{Ground occupied by each plant (cm}^2\text{)}}$$

Chlorophyll content

The leaf sample of crop was made by selecting five healthy fully opened leaves at 45 DAS (50 % flowering) from each plant. The leaves were finely crushed and 20 mg of sample was taken. The pigments from the prepared samples were extracted using dimethyl sulphoxide DMSO extraction method (Hiscox and Israelstam, 1979) by suspending the leaves samples in 5 ml of dimethyl sulphoxide (DMSO) and thereafter, incubating it at 60^{0}C for about one hour in a pre-heated hot air oven. Absorbance of each extract was measured using spectrophotometer at 645 and 663 nm wavelength. The total chlorophyll content was measured using the following equations (Arnon, 1949):

Total chlorophyll content (mg/g) =

$$\frac{(20.2 \times A645) + (8.03 \times A663)}{1000 \times \text{weight of leaf sample taken (g)}}$$

Number of nodules/plant

Five plants were dugout from wet soil condition at 50% flowering stage and then roots were washed gently and thoroughly with water in sieve. The nodules were counted and expressed as number of nodules/plant.

Dry weight of nodules/plant

The root nodules obtained from the selected five plants from each plot was dry under oven at 70^{0}C till a constant weight was recorded on electric weighing machine precisely and then average was worked out as mg/plant.

Soil enzyme

Soil samples were collected at 50 % flowering and were analyzed for dehydrogenase (Klein *et al.*, 1971), urease (Tabatabai and Bremner, 1972) and phosphatase enzyme activity (Tabatabai and Bremner, 1969).

Soil microflora

The enumeration of microbial population (bacteria, fungi and actinomycetes) was done on agar plates containing appropriate media following serial dilution techniques and pour plate method (Collins and Lyne, 1985) and population was recorded as number of cfu per gram of soil.

RESULT AND DISCUSSION

Leaf area index

Leaf area index was recorded at 50 per cent flowering stage and found significant due to various weed control treatments. Significantly higher LAI (5.08) was recorded under two hand weeding over rest of the herbicidal treatments and weedy check. The next best treatments were fomesafen + fluazifop-p-butyl 220 g a.i./ha, propaquizafop + imazethapyr 125 g a.i./ha and sodium acifluorfen + clodinafop-propargyl 245 g a.i./ha. It was found that herbicide mixtures of higher as well as lower dose recorded significantly higher LAI over alone application of herbicides. A perusal of data (Table-2) further indicated that weed control through various herbicides registered higher LAI (2.23-3.97) at 50 per cent flowering compared to weedy crop (1.14). A greater variation in leaf area index of soybean were observed with different weed control treatments. Leaf area index seems to be the function of reduction in weed spread due to these treatments. This could have provided more space for the plants to extend the foliage and branches, thereby providing for more leaves per unit area of land. Similar sort of findings has been reported by Wadafale*et al.* (2011) and Kumar *et al.* (2018).

Chlorophyll content

All the weed control treatments recorded higher chlorophyll content compared to unweeded check (2.15 mg/g) but was non-significant. However, maximum chlorophyll content (2.42 mg/g) at 50 per cent flowering was recorded closely followed by fomesafen + fluazifop-p-butyl 220 g a.i./ha, propaquizafop + imazethapyr 125 g a.i./ha and sodium acifluorfen + clodinafop-propargyl 245 g a.i./ha (Table-2).

Nodules/plant

An insight of data (Table 2) revealed that all the weed control treatments increased the number of nodules/plant significantly over weedy check. Highest nodules/plant

(57.33) was observed with two hand weeding at 20 & 40 DAS which was significantly superior over rest of the treatments. The per cent increase in nodules/plant to the tune of 84.1 over weedy check. The next best effective treatments were application of fomesafen + fluazifop-p-butyl 220 g a.i./ha (52.1), propaquizafop 50 g/ha + imazethapyr 125 g a.i./ha (51.8) and sodium acifluorfen + clodinafop-propargyl 245 g a.i./ha (51.2) in comparison to rest of the herbicidal treatments and weedy check. Sole application of herbicides viz. fomesafen 250 g a.i./ha, imazethapyr 100 g a.i./ha, fluthiacet-methyl 12.5 g a.i./ha, clodinafop-propargyl 60 g a.i./ha, fluazifop-p-butyl 250 g a.i./ha, propaquizafop 50 g a.i./ha and pendimethalin 1.0 kg a.i./ha (PE) were found at par with each other but significantly superior over weedy check. Nodules/plant recorded under two hand weeding, fomesafen + fluazifop-p-butyl 220 g a.i./ha, sodium acifluorfen + clodinafop-propargyl 245 g a.i./ha, propaquizafop + imazethapyr 125 g a.i./ha were 17.6, 151.2, 143.1 and146.2 per cent higher than those observed under weedy check, respectively (Table 2).

Nodules dry weight/plant

Number of nodules/plantin all weed control treatments significantly increased with dry weight of nodules/plant over unweeded check (Table 2). Two hand weeding recorded maximum and significantly higher dry weight of nodules/plant (89.05 mg) over all herbicidal weed control treatments and weedy check. Application of higher dose of herbicide mixture of fomesafen + fluazifop-p- butyl 220 g a.i./ha, sodium acifluorfen + clodinafop-propargyl 245 g a.i./ha andpropaquizafop + imazethapyr 125 g a.i./ha registered maximum nodules dry weight 78.32, 77.61 and 74.58 mg/plant followed by their lower doses (63.85, 63.75 and 64.76 mg/plant). Preemergence as well as post emergence applied herbicides also recorded significantly higher nodules dry weight compared to weedy check. Hence, the treatments having effective weed control provided most favourable conditions for the nodulation and their proper development to attain good weight whereas it was hampered under the treatments, which were infested with weeds. Lokras*et al.* (1985) have also reported increased nodulation under herbicidal treatment.

Soil enzymes activity Dehydrogenase

Two hand weeding at 20 & 40 DAS was recorded maximum dehydrogenase enzyme activity (0.329 µgTPF/g soil/d) closely followed by application of herbicides mixture and alone herbicide while pre-emergence application of pendimethalin + imazethapyr 960 g a.i./ha and pendimethalin 1.0 kg a.i./ha significantly reduced dehydrogenase enzyme activity compared to control. However, the value of dehydrogenase enzyme activity was also higher in weedy check.

Urease

Urease enzyme activity was not much affected under different weed control treatments and weedy check. These treatments were found statistically non-significant and values ranges from 1.16-1.25 µgNH_4N/g soil/d.

Phosphatase

Phosphatase enzyme activity did not influence significantly due to various weed control treatments (Table 2). Though, maximum phosphatase enzyme activity (30.17 µgpnp/g soil/hr) was observed under two hand weeding followed by fomesafen + fluazifop-p-butyl 220 g a.i. /ha.

Generally, soil enzymatic activities were greater in the untreated control which can be ascribed to the greater contribution of weeds and crop stand stimulating production of soil enzymes through microbial and plant origin. Application of post emergence herbicides in soybean did not affect soil dehydrogenase but stimulated urease activity and enhanced yield. This corroborates with the findings of Ramesh*et al*. (2000) and Yogesh (2000).

Soil microflora Total bacteria

All herbicidal weed control measures applied as preemergence and post emergence did not influence total bacterial populations. Though, weedy check recorded maximum bacterial population (22.33 x 10^4cfu/g) followed by two hand weeding at 20 & 40 DAS.

Total fungi

All herbicidal weed control measures could not bring significant variation in total fungi population and found non-significant. However, maximum total fungi population was recorded under weedy check (13.67 x 10^4cfu/g) and two hand weeding at 20 & 40 DAS (13.00 x 10^4cfu/g) and least under pendimethalin + imazethapyr 960 g a.i./ha (10.00 x 10^4cfu/g).

Total actinomycetes

All herbicidal weed control treatments did not bring significant variation in total actinomycetes population and found non-significant. While, maximum total actinomycetes population was recorded under weedy check (12.33 x cfu/g) closely followed by two hand weeding at 20 & 40 DAS (12.00 x 10^4cfu/g).

Since post-emergence herbicides were applied on the foliage of weeds, the amount of herbicide molecules that comes in contact with soil particles were lesser thus their application might have not influence microbial proliferation and activity (Auspurg*et al*., 1989) and Bhimwal *et al*. (2018).

CONCLUSION

The practice of herbicide is a common measure to control weed damage in soybean field. Herbicides affect both soil microbial environment and the action of soil enzymes has been reported. In this research, we applied sixteen herbicides on soybean soil and studied the effects of herbicides on microbial community and soil enzymes activity in the soil of soybean field. Weed control treatments were found statistically superior in improving leaf area index, nodule number and nodule dry weight over weedy check but chlorophyll content was not influenced significantly. Higher LAI, nodule numbers and their dry weight were registered by controlling weeds with two hand weeding followed by ready mix of fomesafen 11.1 % + fluazifop-p-butyl 11.1 % SL @ 220 g a.i./ha, propaquizafop 2.5% + imazethapyr 3.75 % @125 g a.i./ha and sodium acifluorfen 16.5 % EC + clodinafop-propargyl 8 % EC @ 245 g a.i./ha (RM) in comparison to other treatments. Two hand weeding at 20 & 40 DAS was recorded maximum dehydrogenase enzyme activity (0.329 µgTPF/g soil/d) closely followed by herbicides mixture and alone while pre-emergence application of pendimethalin + imazethapyr 960 g a.i./ha and pendimethalin 1.0 kg a.i./ha significantly reduced dehydrogenase enzyme activity compared to control. Pre and post emergence herbicide mixtures and alone did not affect significantly urease and phosphatase enzyme activity in soil and markedly higher values were obtained in herbicidal treatments compared to weedy check. The treatment proceeding soybean crop did not influence the basic microbial activities.

Table 2. Effect of weed control measures on leaf area index, chlorophyll content, nodules/plant, dry weight of nodules/plant, dehydrogenase, urease and phosphatase activity at 50% flowering in soybean

Treatments	Leaf area index	Chlorophyll content (mg/g)	Nodules/ plant (Nos.)	Dry weight of nodules (mg/plant)	Dehydrogenase (µgTPF/g soil/d)	Urease (µgNH_4N/g soil/d)	Phosphatase (µgpnp/g soil/hr)
T1: Weedy check	1.14	2.15	31.14	38.45	0.331	1.19	28.00
T2: 2 HW 20 & 40 DAS	5.08	2.42	57.33	89.05	0.329	1.25	30.17
T3: Pendimethalin 1.0 kg PE	2.30	2.23	39.00	47.56	0.308	1.18	28.81
T4: Imazethapyr 100 g/ha PoE	2.73	2.21	44.00	48.77	0.312	1.28	28.45
T5: Fluthiacet methyl 12.5 g/ha PoE	2.50	2.22	40.00	47.56	0.311	1.34	28.67
T6: Clodinafop-propargyl 60 g/ha PoE	2.25	2.22	39.80	48.88	0.312	1.16	28.53
T7: Fomesafen250 g/ha PoE	3.37	2.35	40.40	48.07	0.313	1.20	28.38
T8: Fluazifop-p-butyl 250 g/ha PoE	3.00	2.28	41.00	48.57	0.311	1.22	28.22
T9: Propaquizafop 50 g/ha PoE	2.23	2.27	39.20	55.65	0.315	1.21	28.30
T10: Pendi. + Imaz. 960 g/ha PE	2.73	2.28	44.40	56.36	0.305	1.18	28.62
T11: Propaqf. + Imaz. 93.7 g/ha PoE	2.87	2.21	42.40	64.76	0.313	1.22	28.67

Table 2 (Continued)

T13:Sod. Acif. + Clodina.F.183.7 g/ha PoE	2.60	2.24	44.40	63.75	0.315	1.24	28.59
T14: Sod. Acif. + Clodina.F.245 g/ha PoE	3.82	2.36	51.20	77.61	0.323	1.23	28.97
T15: Fomsaf. + Fluazi.FB165 g/ha PoE	2.85	2.20	43.80	63.85	0.311	1.23	28.62
T16: Fomsf. + Fluazi.FB 220 g/ha PoE	3.97	2.36	52.10	78.32	0.323	1.22	29.13
SEm ±	0.20	0.068	1.78	1.97	0.004	0.05	1.065
CD (P=0.05)	0.59	NS	5.15	5.69	0.013	NS	NS

Table 3. Effect of weed control measures on soil micro flora in soil at harvest

Treatments	Total Bacteria (10^4 x cfu/g)	Total Fungi (10^4 x cfu/g)	Total Actinomycetes (10^4 x cfu/g)
T1: Weedy check	22.33	13.67	12.33
T2: 2 HW 20 & 40 DAS	21.67	13.00	12.00
T3: Pendimethalin 1.0 kg PE	18.33	10.67	11.00
T4: Imazethapyr 100 g/ha PoE	19.67	11.67	10.67
T5: Fluthiacet methyl 12.5 g/ha PoE	20.33	11.67	11.67
T6: Clodinafop-propargyl 60 g/ha PoE	19.67	12.00	12.00
T7: Fomesafen250 g/ha PoE	20.67	11.00	10.67
T8: Fluazifop-p-butyl 250 g/ha PoE	20.67	12.00	11.67
T9: Propaquizafop 50 g/ha PoE	20.00	12.00	11.33
T10: Pendi. + Imaz. 960 g/ha PE	17.67	10.00	10.33
T11: Propaqf. + Imaz. 93.7 g/ha PoE	21.00	11.33	11.67
T12: Propaqf. + Imaz.125 g/ha PoE	20.33	12.33	11.67
T13:Sod. Acif. + Clodina.F.183.7 g/ha PoE	20.00	11.67	10.33
T14: Sod. Acif. + Clodina.F.245 g/ha PoE	20.33	12.00	11.00
T15: Fomsaf. + Fluazi.FB165 g/ha PoE	21.00	12.33	11.33
T16: Fomsf. + Fluazi.FB 220 g/ha PoE	20.33	11.67	11.00
SEm ±	0.81	0.61	0.43
CD (P=0.05)	NS	NS	NS

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Inheritance of seed storability using accelerated aging test in soybean [*Glycine max* (L.) Merr]

AMIT T ADSUL[1], VIVEK P CHIMOTE[2], DILIP S THAKARE[3], MILIND P DESHMUKH[4]

[1]Department of Genetics and Plant Breeding, MPKV, [2]Department of Botany, State Level Biotechnology Centre, MPKV, [3]Department of Genetics and Plant Breeding, MPKV, [4]Agriculture Research Station, Sangli

Email: amitadsul444@gmail.com

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ABSTRACT

Soybean is known for the poor seed germination particularly in the tropical and subtropical country like India. However, the molecular mechanisms involved are mostly unknown in these species. The proportion of seed coat is of primary importance for providing protection against mechanical damage during harvesting and processing which cause significant loss of seed longevity in soybean seed lot. The present investigation finds the black seeded genotypes viz., Birsa Soya1 and Kalitur had significantly higher amount of seed coat lignin than other yellow seeded genotypes viz., DS-228 and MAUS-71. The crosses between black and yellow coloured to produce different colour pigmentation in their progeny viz., black, brown, buff and yellow. However, seed coat lignin is highly positively correlated with seed longevity. The seed coat lignin was transfer to yellow seeded genotypes through backcrossing. The red colour flavanoids were found to be concentrated in the seed coat (testa) just outside the aleurone layer in the cross of DS-228 × Birsa Soya1. The seed coat (testa) is a maternal trait which is influence genotypes of the mother plants. Furthermore, tannins reacting with vanillin-HCl were demonstrated in the testa of soybean. However, the inheritance of red colour was observed to genetically transfer to their progeny. These results provide essential information to explain the poor germination observed in soybean genotypes and it can improve through the molecular breeding.

Keywords: Correlation; Inheritance; Seed coat lignin; Seed longevity; Soybean

Soybean [*Glycine max* (L.) Merrill] is an important legume crop belongs to family leguminacae, subfamily papilionaceae and genus glycine. It has been domesticated from *Glycine soja* Sieb & Zucc. in northeast China where first written record dated back to 2328 B.C. (Hymowitz et al. 1980). It has been cultivated at broadly diverse geographical locations and under many different-growing conditions, particularly in the America and Asia. As per AMIS, FAO estimates, among the major soybean growing countries, India ranks fourth in terms of area and fifth in terms of production. During 2020–21, soybean was grown in an area of 12.06 million hectare with a production of 13.58 million tons and productivity of 1126 kg/ha. Indian soybean productivity has stagnated around 1200 kg/ha while world soybean productivity stands at 2900 kg/ha and the USA and Brazil are the countries with highest productivity (> 3000 kg /ha) (AMIS, FAO website). Soybean is an introduced crop and very sensitive to photoperiod and temperature. Impact of climatic adversities in recent past had a severe impact to harvest soybean to its full potential even though improved and high yielding varieties are mostly grown in major soybean growing areas.

Soybean seed is inherently prone to loss of viability and has been grouped as least storable among all the grain crops (ISTA 1999). Physiological maturity soybean seed reaches its maximum potential for germination and vigour (Sung et al. 1996). This potential is short lived as compared to other grain crops and is often reduced prior to planting time (Nagai 1921). The reason for short life of soybean seed is its susceptibility to high temperature and humidity, which makes production of quality seeds and maintenance of its vigour more difficult in tropical countries as compared to temperate environment (Dassou et al. 1984). Field weathering of soybean seed is by far the most crucial factor responsible for poor quality of seed harvested under tropical conditions followed by faster rate of seed deterioration during storage and mechanical damages during harvesting and post-harvest handling. Also, the rate of seed deterioration during storage is very high for field-weathered seeds as compared to unweathered seeds. Genotypic variation in response to field weathering has been reported by several workers (Nagai 1921). Environmental factors are also known to influence seed quality during its development, desiccation period (physiological maturity to harvest maturity) and beyond harvest maturity when the seed is essentially attached to mother plant in the field itself (Dimov et al. 2012 and Justice et al. 1978).

Seed coat lignin is nature's most abundant biopolymer in vascular plants. It is a complex polymer of hydroxylated and methoxylated phenylpropane units. It is a major constituent of cell walls and provides these cells with rigidity for structural support and impermeability to water (Bay et al. 1995 and Franca et al. 1984). There is considerable interest in lignin since its deposition in the seed coat provides mechanical resistance (Piper et al. 1923). Soybean seed coat is very thin and low in lignin content and provides little protection to the fragile radicle which lies in a vulnerable position directly beneath the seed coat (Agrawal et al. 1974 and Egli et al. 2005). Due to this fact, mechanical damage is the most important factor that reduces soybean seed quality at harvest and processing (Costa et al. 1987). To manage the problem of rapid seed deterioration in soybean, it is imperative to understand the basic mechanisms involved. Several mechanisms such as impairment of membrane function, inhibition of protein synthesis, decline in sugar content, damage to enzyme systems, damage to nuclear material including RNA, DNA and chromosomes and biochemical changes resulting in lower levels of ATP have been suggested for loss of seed vigour (Kuchlan et al. 2010). In recent years, growing evidences point to the toxicity of by-products of catabolic reactions such as lipid peroxidation, modification of proteins and sugars thorough Amadori and Millard reaction resulting in deterioration of seeds (Kuchlan et al. 2010; Smith et al. 1952 and Sun et al. 1995).

To study the inheritance of the yellow seed character in relation to seed germination, seed longevity, pre-harvest germination and other seed quality traits. The seed coat controls seed germination and can be seen as a barrier for water and/or oxygen, or by providing mechanical resistance to radicle protrusion. The significant advantages of yellow seed coat, the thinner testa in yellow-seeded types also means the seed is more prone to damage by various environmental factors. Significant genetic variations for lignin content and bimodal frequency distributions were found in both populations. Multiple genes and loci have been reported to be involved in the inheritance of seed coat color and testa thickness not only in Arabidopsis thaliana but also in oilseed species. The newly identified QTL for reduced lignin content on chromosome C03 is valuable for studying additive and epistatic effects in combination with other low lignin genotypes. SNP marker segregation in contrasting bulks revealed for both populations overlapping narrow genomic regions on chromosome C03, responsible for reduced lignin content. Correlations of acid detergent lignin content to seed coat phenolic compounds suggest that low acid detergent lignin content is associated with reduced seed coat thickness (Widiarsih et al. 2021).

Seed longevity decline also during dry storage and the aging rate depends on seed moisture contents, temperature, initial seed quality and on genetic factors. Seed longevity of yellow-seeded types tends to drop more rapidly compared to the black-seeded one. Since materials of naturally aged seeds are not always available, artificial seed aging protocols are often utilized. Exposure of seeds to high temperature and high moisture conditions has been a commonly used method for aging seeds in the laboratory. To study the inheritance of the yellow seed trait in relation to seed germination and other seed quality traits. For this purpose, characterized yellow seeded varieties were crossed to the same black seeded varieties and derived segregating populations were characterized field experiments (Widiarsih et al. 2021).

MATERIALS AND METHODS

The present investigation entitled "Inheritance of seed storability using accelerated aging test in soybean (*Glycine max* [L.] Merrill)" was conducted at Post Graduate Institute, Botany Research Farm and Seed Technological Research Unit, MPKV, Rahuri. Evaluation of experimental field trial conducted by using randomized complete block design (RBD) statistical design with three replications. The seeds of four genetically diverse genotypes along with pedigree and salient features (Table 1) were obtained from the Soybean Breeder, Agricultural Research Station, Kasbe Digraj, Sangli (MH) India. Statistical analysis was carried out by using experimental design *viz.*, randomized block design along with three replication on evaluation trails.

Accelerated aging test was conducted by using the jar accelerated ageing system. Seeds were placed in muslin cloth bags tied with rubber band and placed on seed germinated chamber. There were approximately 100% relative humidity and incubator at 42°C for 72 hours. Each treatment consisted of 25 seeds each replicated four times, placed in seed germinated chamber (Kuchlan et al. 2010). After ageing, standard germination test was conducted and the normal seedlings were counted on 7th day as stated in (ISTA 1999).

Germination test: The two replication of 25 seeds each four crosses of six generations placed on germination paper (between paper method by ISTA) and stored at seed germinated chamber for 25°C temperature and 80% relative humidity and counted germination percentage after 7 days (ISTA 1999).

Seed coat lignin: Near Infrared Reflectance Spectroscopy (NIRS) was applied to predict moisture, oil, protein, lignin and glucosinolate content of the seed, and fiber contents of the defatted meal. NIRS measurements were performed using seed samples of 3g in small ring cups and the FOSS monochromator model 6500 (NIRSystem Inc., Silverspring, MD, USA). Oil, protein, lignin and glucosinolate (lmol/g seed) contents were predicted at 91% seed dry matter content using the commercial calibration equations of raps 2012.eqa provided by VDLUFA Qualita¨tssicherung NIRS GmbH (Am Versuchsfeld 13, D-34128 Kassel, Germany). Total seed oil and protein content was obtained by forming the sum of oil and protein content. The values of oil and protein content were further used to calculate protein content of defatted meal (PDM): PDM (%) = [% protein/(100% oil)] 9 100. NDFm, ADFm and ADLm contents were determined by calibration equations fibr2013.eqa (Dimov et al. 2012). The lignin content in soybean seed was estimated on ZEUTEC Spectra Analyzer (A dual beam near infrared spectrometer). In the spectra analyzer, the sample was exposed to near infrared light of specific wavelengths, selected from up to 19 high precision interference filters. The lights penetrates the sample, interact with sample molecules and was partly absorbed and partly diffusely reflected. The reflected light was measured by a lead sulfide (PbS) detector mounted in a gold coated integrating sphere located following the sample (Thimmaiah S.R. 1999) (Table 3). The amount of light absorbed by the sample at different wavelengths was directly related to the concentration of chemical functional groups *viz.* C-H, O-H and N-H.

Table 1. Pedigree and salient features soybean genotypes:

Genotypes	**DS-228**	**MAUS-71**	**Kalitur**	**Birsa Soya1**
Pedigree	JS-335 x Ankur	Identified	Local Selection	Mutant of sepaya black
Developed by	MPKV, Rahuri	MAU, Parbhani	NRC, Indore	BAU, Ranchi
Stem termination	Determinate	Semi-Determinate	Semi-determinate	Determinate
Pubescence	Absent	Absent	Present	Present
Pubescence colour	Absent	Absent	Medium tawny	Tawny
Flower colour	Violet	Violet	Dark Violet	White
Hilum colour	Brown	Black	Black	White
Days to flowering	Early	Early	Medium	Late
Days to maturity	Early	Early	Late	Late
Seed size	Large	Medium	Small	Large
Germination	Poor	Poor	Very good	Very good
Accelerated aging	53.60% (Low)	54.54% (Low)	82.88% (High)	82.40% (High)

Table 2. Parental details of different generations of the crosses:

Generation	Cross-I	Cross-II	Cross-III	Cross-IV
P1	DS-228	DS-228	MAUS-71	MAUS-71
P2	Kalitur	Birsa Soya1	Birsa Soya1	Kalitur
F1	DS-228 × Kalitur	DS-228 × Birsa Soya1	MAUS-71 × Birsa Soya1	MAUS-71 × Kalitur
F2	Self of F_1	Self of F_1	Self of F_1	Self of F_1
B1	(DS-228 × Kalitur) × DS 228	(DS-228 × Birsa Soya1) × DS-228	(MAUS-71 × Birsa Soya1) × MAUS-71	(MAUS-71 × Kalitur) × MAUS-71
B2	(DS-228 × Kalitur) × Kalitur	(DS-228 × Birsa Soya1) × Birsa Soya1	(MAUS-71 × Birsa Soya1) × Birsa Soya1	(MAUS-71 × Kalitur) × Kalitur

As these concentrations are in turn related to the concentrations of the properties of interest, *e.g.* lignin, fats, oil, protein or fatty acids, property values can be determined. Before the instrument can be used to analyze samples, it must be calibrated, using a representative set of similar samples of the product to be analyzed and their known reference values. These values must have been obtained by using standard laboratory methods of high accuracy. In a calibration, a correlation between the NIR absorbance values at the different wavelengths and the sample composition was established and calibration constants are calculated for each wavelength.

Table 3. Total number of samples for seed coat lignin content:

Sr. No.	Generations	Experimental Materials				Total
		DS 228 × Kalitur	DS 228 × Birsa Soya1	MAUS 71× Birsa Soya1	MAUS 71 × Kalitur	
1.	P_1	1	1	1	1	4
2.	P_2	1	1	1	1	4
3.	F_1	1	1	1	1	4
4.	F_2	1	1	1	1	4
5.	B_1	1	1	1	1	4
6.	B_2	1	1	1	1	4
Grant Total		6	6	6	6	24

Proportion of seed coat: The seed coat from 20 seed of each four crosses of six generations were removed carefully with the help of forceps after wetting sample of each crosses for 3 hours in petridish and then drying at room temperature for 3 days. To the weight of cotyledon and seed coat weighing separately and calculated proportion of seed coat percentage (Keigley et al. 1986) by following formula,

$$\frac{\text{Weight of seed coat} \times 100}{\text{Weight of seed coat and cotyledons}}$$

Seed density: It was calculated to the volume of seed was measured following volume expansion principle. A 200 ml volumetric flask was filled with toluene up to the level of 50 ml and a 5-g seed was added in to it. The increase in volume was measured and density of seed was calculated as weight of whole seed per unit volume (Keigley et al. 1986).

Seed coat permeability: The weight of dry seed sample 5 gm of each four crosses of six generations in two replications on weighing balance machine. Then the simultaneously same weight of each four crosses of six generations placed in petridish 10 hours with water content on it for. Then take weight of imbibe seed of four crosses. Seed coat permeability was negatively correlated with seed longevity (Keigley et al. 1986).

Seed coat colour of populations in soybean were observed phenotypic (Fig 3) (Mugnisjah et al. 1987 and Nkang et al. 1997) and their classifications as per the following (Table 4).

Table 4. Classification of seed coat colour:

Self-color type	**Bicolor type**	**Eyebrow pattern with green or yellow background**	**Green or yellow seed coat with dark hilum**	**Green or yellow seed coat with light hilum**
Black	Black mottling with brown	Black	Hilum black	Mottled with black
Brown	-	Brown	Hilum brown	Mottled with brown
Imperfect black	-	Imperfect black	Hilum imperfect	Mottled with imperfect black
Buff	-	Buff	Hilum buff	Mottled with buff

Tetrazolium Test**:** It was used for quick estimate of the viability of seed samples through biochemical reaction. The imbibed chemical in living plant tissues is reduced from a colorless solution to red insoluble compound, which precipitates in live cells, while dead cells remain colorless because of no reaction. Three replications of 50 seeds each four crosses of six generations. The treatments were preconditioned between folded moist paper towels for 18-20 hours, in order to allow slow water uptake to avoid cotyledon cracking. After preconditioning the seeds were removed and each lot was divided into two halves of 50 seeds each. The seeds were then immersed in 1% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) in one liter distilled water of PH. 7.0 at 35°C for 3-4 hours to allow staining, according to the procedure adopted by (Grabe D.F. 1970). After staining, the solution was decanted and the seeds were rinsed several times in tap water, put in glass beaker and refrigerated in moist condition and evaluated immediately. After removing the seed coat, each seed was cut longitudinally through the embryo axis into two halves and evaluated under a dissecting microscope. In addition to completely stained viable seeds and completely unstained non-viable seeds, some were partially stained. However seeds were separated in the viable and non-viable seeds according to (ISTA 1999). The number of viable seeds in each treatment was determined and average percentage was calculated (Fig 4).

***Vanillin Test*:** The twenty seed or aborted seeds of each crosses were hand cut in halve of replication wise and placed for half hour in 1% vanillin-6 M-HCl solution which stains proanthocyanidis and catechins yielding an intense red colour. After staining half seeds were washed in distilled water and stored at -20^{0}C. Stained and unstained half seeds were in spected at low magnification. Thin section (10 um) were then made on a new blade and transferred to moist gelatin-coated slides before examination ah higher magnification in a Reichert Univar microscope (Reichert Austria) (Aastrup et al. 1984). Photographs were taken on Kodak Ektachrome 450 film (Kodak USA) (Fig 5).

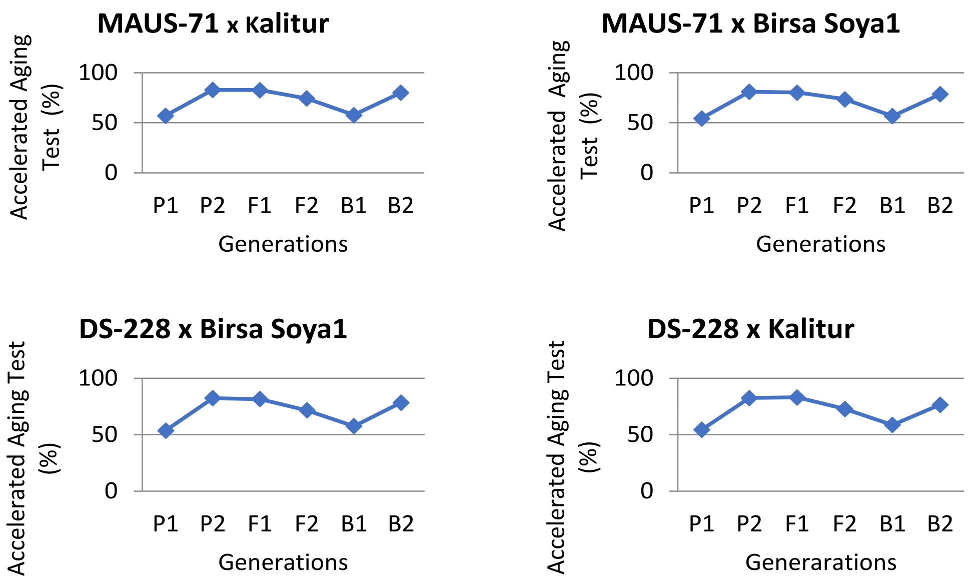
RESULTS AND DISCUSSION

The present investigation entitled "Inheritance of seed storability using accelerated aging test in soybean (*Glycine max* [L.] Merrill)". Inheritance of seed quality traits and correlation coefficient between seed quality related traits in soybean genotypes *viz.,* high seed longevity genotypes (Birsa Soya1 & Kalitur) and low seed longevity genotypes (DS-228 & MAUS-71) were crossed in manner (Low × High) (Table 1). The significant genotypic variation within segregating population was detected for all seed quality traits without and with artificial aging treatments. The inheritance of seed quality traits content ranged from *viz.,* seed coat lignin (0.44 to 1.26); accelerated aging (53.60 to 83.09); germination percentage (69.33 to 96.66); proportion of seed coat (8.10 to 11.35); seed density (1.06 to 1.11); and seed coat permeability (6.08 to 9.78). The segregating population had a significantly higher content of all seed quality traits as compare to parental.

Inheritance of seed quality related traits in soybean:

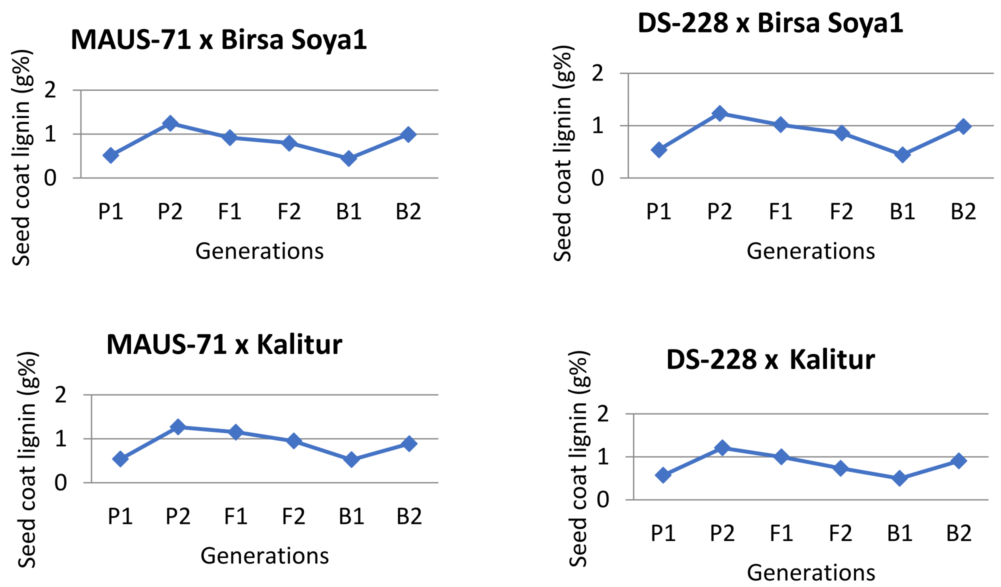
Accelerated aging test was conducted at laboratory using sample of parents and segregating population of all four crosses. The varietal differences in seed vigour were clearly demonstrated of accelerated aging ranged from 53.60 to 83.09% (Fig 1) (Table 5). The present study was similar to accelerated aging test in soybean as proposed for predicting the storability of seed lot (Baskin C.C. 1981 and Delouche et al. 1973). The results show on accelerated aging for all four crosses that varietal difference in seed vigour were clearly demonstrated due to the inheritance ability. According to results it's concluded that the accelerated aging in soybean genotypes is transfer from parent to their segregating population.

Fig 1. Accelerated Aging Test (AAT):



Seed coat lignin content was ranging from four crosses *viz.,* 0.4446 to 1.2694 (g%) (Table 5) (Fig 2). Black seeded varieties *viz.,* Birsa Soya1 and Kalitur had significantly higher amount of lignin than other yellow seeded varieties. The lignin content trait was heritable and transfer to through backcrossing programme. Lignin is known to import mechanical strength to seed coat (Alvarez et al. 1997). The higher the lignin content in the seed coat, the greater was the expected resistance to mechanical damage. This resistance is genetic characteristic that varies among soybean cultivars (Carbonell et al. 1995). Black seeded varieties *viz.,* Birsa Soya1 and Kalitur had significantly higher amount of lignin than other yellow seeded varieties. The seed coat lignin was transfer to yellow seeded varieties through backcrossing.

Fig 2. Seed Coat Lignin:



The proportion of the seed coat content was ranging from (8.10 to 11.35%) in all four crosses (Table 5). The black seeded varieties, Birsa Soya1 and Kalitur, had highest seed coat to seed proportion. The proportion of the seed coat is observed as a heritable trait in all four crosses which is primary importance in providing protection against mechanical damage during harvesting and processing, which cause significant loss in viability of seed lot. The black seeded varieties, Birsa Soya1 and Kalitur, had highest seed coat proportion trait to which was transfer through crossing progamme in yellow-seeded varieties. The proportion of the seed coat is of primary importance in providing protection against mechanical damage during harvesting and processing, which cause significant loss in viability of seed lot.

The seed density content was ranging from 1.0684 to 1.1182 g/cm^3. There was significant inheritance for seed density among different four crosses. The black seeded varieties good stored *viz.*, (Birsa Soya1 and Kalitur) and inherited to its yellow seeded varieties through crossing programme (Table 5). The lower seed density has also been associated with lower seed quality (Hosamani et al. 2013 and Hoy et al. 1985). There was significant inheritance for seed density among the all four crosses. The black seeded varieties good stored *viz.*, (Birsa Soya1 and Kalitur) and inherited to its yellow seeded varieties through crossing programme.

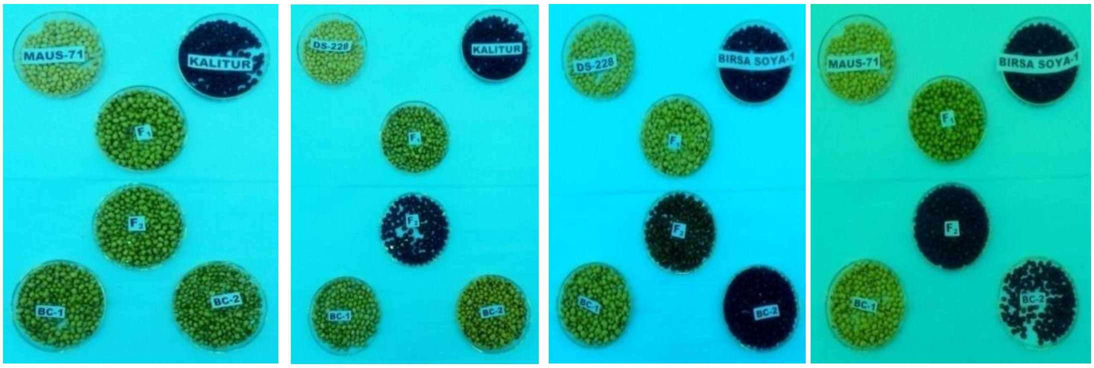
Seed coat permeability of segregating population in soybean was observed in all four crosses. The seed coat permeability ranging from (6.08 to 9.78g) in all four crosses (Table 5). The seed coat permeability trait was heritable and directly proportional to the seed coat lignin. The high seed coat permeable there is low proportional to seed coat lignin and less proportional of seed coat permeable there is greater chance of high seed coat lignin in soybean seed. The four independent markers (Satt434, Satt538, Satt281 and Satt598) were significantly (P=0.05) associated with seed coat permeability (Widiarsih et al. 2021).
The inheritance of seed coat colour trait was observed phenotypic in all four crosses of parents as well as their populations (Fig 3). The crosses between black and yellow colour to produce different colour pigmentation in their progeny *viz.*, black, brown, buff and yellow. However, phenotypic seed coat colour trait was linked to some extent with desired trait of seed longevity. In soybean segregation population of (F3'H & F3'5'H) was strongly influence the flower and seed coat coloration (Grabe D.F. 1970). Two major genes with additive, dominance and epistasis effect were responsible for controlling seed coat colour trait in sesame. Alleles at all QTL's from the black seeded parent tended to increase the seed coat colour (Tekrony et al. 1987). The black seed coat colour has been associated with resistance to field weathering resistance against pathogen and hence better storability (McDonald M.B. 1999). The crosses between black and yellow coloured to produce different colour pigmentation in their progeny viz., black, brown, buff and yellow. However, seed coat colour is linked to some extent with desired trait of seed longevity.

Table 5. Descriptive statistics (ANOVA) of seed quality traits in soybean

Crosses	Traits	P1	P2	Min	Max	Mean	SD	LSD 5%
DS-228 x Kalitur	Seed Coat Lignin (g%)	0.57	1.21	0.49	1.21	0.82	0.27	0.90
	Accelerated aging test (%)	54.26	82.56	54.26	83.09	71.29	12.22	8.41
	Germination (%)	69.33	95.33	69.33	95.33	84.11	11.06	9.13
	Proportion of Seed Coat (%)	8.77	11.35	8.10	11.35	9.47	1.27	3.06
	Seed Density (g/cm^3)	1.09	1.11	1.07	1.11	1.09	0.02	1.04
	Seed Coat Permeability (g)	9.14	6.12	6.11	9.14	7.69	1.32	2.76
DS-228 x Birsa Soya1	Seed Coat Lignin (g%)	0.54	1.23	0.44	1.23	0.85	0.30	0.92
	Accelerated aging test (%)	53.60	82.40	53.60	82.40	70.87	12.49	8.38
	Germination (%)	70.66	90.66	70.66	90.66	81.10	8.46	8.96
	Proportion of Seed Coat (%)	8.57	10.09	8.28	10.09	8.99	0.68	2.98
	Seed Density (g/cm^3)	1.10	1.12	1.09	1.11	1.10	0.01	1.04
	Seed Coat Permeability (g)	9.12	6.11	6.13	9.12	7.78	1.36	2.77
MAUS-71 x Birsa Soya1	Seed Coat Lignin (g%)	0.52	1.25	0.45	1.25	0.82	0.30	0.90
	Accelerated aging test (%)	54.54	81.12	54.54	81.12	70.83	12.09	8.38
	Germination (%)	70.66	88.66	70.66	91.33	81.55	8.34	8.99
	Proportion of Seed Coat (%)	8.91	10.57	7.94	10.57	9.04	0.89	2.99
	Seed Density (g/cm^3)	1.09	1.12	1.09	1.12	1.10	0.01	1.04
	Seed Coat Permeability (g)	9.78	6.12	6.08	9.78	7.87	1.50	2.79
MAUS-71 x Kalitur	Seed Coat Lignin (g%)	0.54	1.27	0.52	1.27	0.89	0.31	0.93
	Accelerated aging test (%)	57.16	82.88	57.16	82.88	72.51	12.04	8.47
	Germination (%)	69.33	96.66	69.33	96.66	84.22	10.76	9.13
	Proportion of Seed Coat (%)	8.69	11.03	8.48	11.03	9.54	1.11	3.07
	Seed Density (g/cm^3)	1.09	1.12	1.08	1.12	1.10	0.01	1.04
	Seed Coat Permeability (g)	9.73	6.16	6.16	9.73	7.66	1.37	2.75

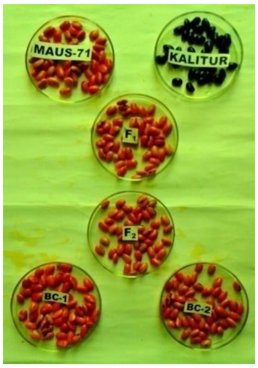
T-distribution value=1.7247; *Seed coat colour*, *Tetrazoliun Test* and *Vanillin Test* (Fig 3, 4, 5) resp.

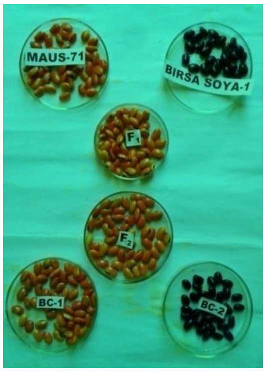
Fig 3. Seed Coat Colour:

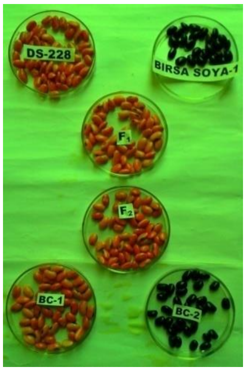


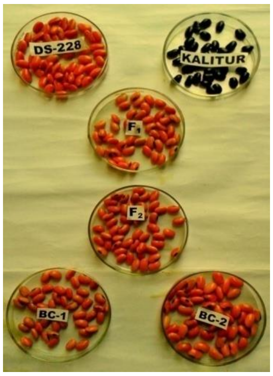
MAUS-71 × Kalitur DS-228 × Kalitur DS-228 × Birsa Soya1 MAUS-71 × Birsa

The inheritance of seed viability was conducted by using tetrazolium (TZ) test in all four crosses (Fig 4). The viability evaluation involves the identification, location and appraisal of sound, weak and dead embryo tissue relayed to seedling development, overall strength of the development seedling and possible influence on length of life of the seed in storage. Estimate both seed germination and viability for an estimation of germination, stained seed are placed in a germinable or non-germinable category based on the overall colour, location and amount of dead tissue (Delouche et al. 1962). The reaction was catalyzed by diphosphopyridine nucleotide linked dehydrogenase in malic and alcohol systems and was mediated by diaphorase (Shimamoto Y. 2001). According to results phenotypic observation of TZ test conducted in parental and segregation populations which was a heritable because this test shows similar results in parents and their progenies. These results showed TZ test was highly correlated with the germination percentage in soybean. The viability evaluation involves the identification, location and appraisal of sound, weak and dead embryo tissue relayed to seedling development, overall strength of the development seedling and possible influence on length of life of the seed in storage.







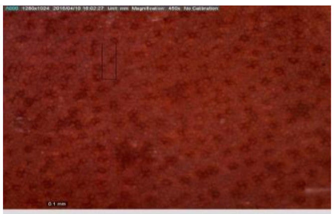


MAUS-71 × Kalitur | MAUS-71 × Birsa Soya1 | DS-228 × Kalitur | DS-228 × Birsa Soya1

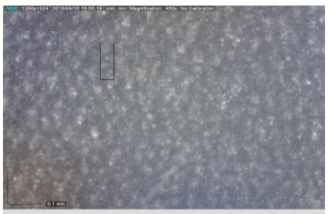
Fig 4. Tetrazolium Test:

Vanillin test was conducted in parents of soybean seed and their progenies in all four crosses, it showed heritable and because it reaction similar reaction observed in their segregation population (**Fig 5**). A vanillin-HCl staining was used to locate the proanthocyanidins in mature soybean seeds of segregation population of cross DS-228 × Birsa Soya1. These flavanoids were found to be concentrated in the seed coat (testa) just outside the aleurone layer in this cross with red coloured seeds investigated. Furthermore, tannins reacting with vanillin-HCl were demonstrated in the testa of soybean. The inheritance of red colour was observed to genetically transfer to their progeny. The inheritance of red colour was observed to genetically transfer to their progeny.

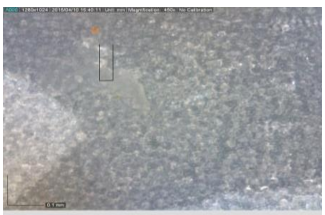
DS-228



Birsa Soya1

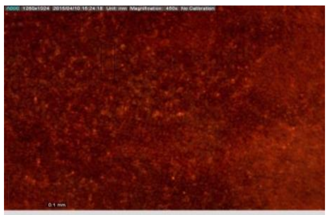


F1



F2

BC1



BC2

Fig 5. Vanillin Test:

Correlation coefficient between seed quality related traits in soybean:

Correlation studies are important in plant breeding, as emphasized the use of this index to describe the degree of association between two or more traits (Galton F. 1888). It gives the total mutual relationship between two traits. When two variables change together in such a way that an increase in one variable is accompanied by an increase in the other, the variables are said to be *positively correlated*. In biological measurements, the relationship between the two variables is not likely to be as complete as this, but it is obvious that certain characters may be expected to show a strong correlation. Should an increase in one variable go hand in with a decrease in the other, these two variables are said to be *negatively correlated*. If there is no relationship between two variables, they are said to be independent or uncorrelated. Correlation coefficients between seed quality traits in soybean have been presented follow (Table 6); Seed coat lignin is highly *positively correlated* with accelerated aging test (0.926**), (0.962**), (0.933**) & (0.948**) in the crosses of DS-228 × Kalitur, DS-228 × Birsa Soya1, MAUS-71 × Birsa Soya1 and MAUS-71 × Kalitur respectively. Seed coat lignin is highly *positively correlated* with germination percentage (0.937**), (0.972**), (0.869**) & (0.985**) in the crosses of DS-228 × Kalitur, DS-228 × Birsa Soya1, MAUS-71 × Birsa Soya1 and MAUS-71 × Kalitur respectively (Fig 1). Accelerated aging test is highly *positively correlated* with germination percentage (0.995**), (0.974**), (0.964**) & (0.962**) in the crosses of DS-228 × Kalitur, DS-228 × Birsa Soya1, MAUS-71 × Birsa Soya1 and MAUS-71 × Kalitur respectively (Table 6).

Proportion of seed coat is highly *positively correlated* with accelerated ageing test (0.778**), (0.802**) & (0.805**) in the crosses of DS-228 × Kalitur, DS-228 × Birsa Soya1 and MAUS-71 × Kalitur respectively. Proportion of seed coat is highly *positively correlated* with germination percentage (0.813**), (0.836**), (0.498*) & (0.755**) in the crosses of DS 228 × Kalitur, DS-228 × Birsa Soya1, MAUS-71 × Birsa Soya1 and MAUS-71 × Kalitur respectively. Germination percentage is highly *positively correlated* with accelerated aging test (0.995**), (0.974**), (0.964**) & (0.962**) in the crosses of DS-228 × Kalitur, DS-228 × Birsa Soya1, MAUS-71 × Birsa Soya1 and MAUS-71 × Kalitur respectively (Table 6). Seed density is *positively correlated* with accelerated ageing test (0.516*), (0.556*) & (0.738**) in the crosses of DS-228 × Birsa Soya1, MAUS-71 × Birsa Soya1 and MAUS-71 × Kalitur respectively. Seed density is *positively correlated* with germination percentage (0.632**) & (0.720**) in the crosses of MAUS 71 × Birsa Soya1 and MAUS 71 × Kalitur respectively (Table 6). Seed coat permeability is highly *negatively correlated* with accelerated ageing test (-0.777**), (-0.807**), (-0.769**) & (-0.885**) in the crosses of DS-228 × Kalitur, DS-228 × Birsa Soya1, MAUS-71 × Birsa Soya1 and MAUS-

71 × Kalitur respectively. Seed coat permeability is *highly negatively correlated* with germination percentage (-0.763**), (-0.726**), (-0.655**) & (-0.855**) in the crosses of DS-228 × Kalitur, DS-228 × Birsa Soya1, MAUS-71 × Birsa Soya1 and MAUS-71 × Kalitur respectively. The high and significant *positive correlation* (r=0.7284) between accelerated aging and seed quality related traits in soybean (Hosamani et al. 2013). It was evident that the genotype differed significantly with respect to storability. Seed coat permeability and electrolyte leaching are the important traits that have been *negatively associated* with accelerated aging in soybean (Widiarsih et al. 2021).

Table 6. Correlation Coefficient:

Traits	Accelerated ageing test (%)				Germination percentage			
	DS228 × Kalitur	DS228 × Birsa Soya1	MAUS71 × Birsa Soya1	MAUS 71 × Kalitur	DS228 × Kalitur	DS228 × Birsa Soya1	MAUS71 × Birsa Soya1	MAUS71 × Kalitur
Seed coat permeability (g)	-0.777**	-0.807**	-0.769**	-0.885**	-0.763**	-0.726**	-0.655**	-0.855**
Seed Density (g/cm3)	0.181	0.516*	0.556*	0.738**	0.145	0.543	0.632**	0.720**
Proportion of seed coat (%)	0.788**	0.802**	0.465	0.805**	0.813**	0.836**	0.498*	0.755**
Seed coat lignin (g%)	**0.926****	**0.962****	**0.933****	0.948**	0.937**	0.972**	0.869**	0.985**
Accelerated aging test (%)	1.000	1.000	1.000	1.000	0.995**	0.974**	0.964**	0.962**
Germination percentage	0.995**	0.974**	0.964**	0.962**	1.000	1.000	1.000	1.000

*Significant at 5 % (0.468) and ** Significant at 1 % (0.590).

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Materials & Methodology preparation, data collection and analysis were performed by [Amit T. Adsul], [Vivek P. Chimote] [Dilip S. Thakare] and [Milind P. Deshmukh]. The first draft of the manuscript was written by [Amit T. Adsul] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Establishment of an Efficient Regeneration System Using Embryonic Tip as anExplant

SHRUTI SHUKLA[1], ANITA RANI[1*], MEETA JAIN[2], VINEET KUMAR[1]

[1]ICAR- Indian Institute of Soybean Research, Indore, Madhya Pradesh, India, [2] School of Biochemistry, DAVV, Indore, Madhya Pradesh, India

Email: anitavka@yahoo.co.in

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ABSTRACT

An efficient and reproducible plant regeneration system from transformed embryonic tip explants was established by the right choice of carbohydrate and the optimum concentration of phytohormones. In this study, we evaluated the effect of different carbohydrates i.e., sucrose, maltose, or sorbitol, along with the phytohormones, i.e., 6-benzyl adenine (6-BA) and indole butyric acid (IBA) on shootregeneration frequency and subsequently analyzed the effect of gibberellic acid (GA3), and indole acetic acid (IAA) on shoot elongation rate. Maltose was shown to be the best carbohydrate source, while 0.2 mg/l and 0.4 mg/l of 6-BA and IAA were found to be the optimal concentrations in shoot induction (SI) media, respectively. The average shoot elongation rate (an average 6.3 cm shoot length along with multiple shoots) is significantly enhanced by the addition of 1.5 mg/l IAA and 0.2 mg/l GA3. This study provides an improved regeneration frequency and subsequently Agrobacterium-mediated transformation efficiency in soybean.

Keywords: Soybean, Regeneration Efficiency, Carbohydrate Source, Plant Growth Regulators, Transformation Efficiency

The efficiency of T-DNA transfer and the frequency of shoot regeneration are thetwo most critical events which affect the successful *Agrobacterium* - mediated transformation efficiency using any of the explant systems. Soybean transformation remains challenging due to the absence of an appropriate competent target for infection and the lack of an efficient regeneration system after transformation. Therefore, changes in culture conditions are required to resolve issues related to low transformation efficiency and a slow regeneration process. In this regard, a proper regeneration system is needed to overcome the low shoot induction after transformation by altering culture conditions and media supplements. Rate and mode of shoot multiplication are the two major elements that determine the success of regeneration. An active and fast regeneration procedure is required that may regenerate more plantlets in a short period. Factors that affect the shoot regeneration include the genotype of the plant, growth medium, status of the media, growth regulators, organic and inorganic elements, explant types, the composition of phytohormones, and ethylene inhibitors, such as $AgNO_3$, $Ag_2S_2O_3$, amino ethoxy- vinyl-glycine, and proline. Since *in vitro* plant cells, tissues, and organ cultures are not fully autotrophic, carbohydrates are required in culture media to maintain the osmotic potential as well as to provide energy and carbon for several highly energy-consuming developmental processes such as shoot proliferation, root induction, and emission, embryogenesis, and organogenesis (Yaseen *et al* 2013). Based on genotypes and particular growth stages, different carbon sources have been employed in culture media. Significant sources of carbohydrates that plants can use include the monosaccharide hexoses (glucose, fructose, galactose, and mannose), pentoses (arabinose, ribose, xylose), disaccharides (maltose, lactose, cellobiose, trehalose), and trisaccharide (raffinose), aswell as sugar alcohols (alternative carbohydrates) like sorbitol (George and Hall 2008). Moreover, the higher transformation efficiency with the media containing sugarproved the synergistic role of carbohydrates.

Successful plant transformation is also affected by PGR, and competence for transformation can be enhanced by PGR treatments (Villemont *et al* 1997). An explant becomes susceptible to *Agrobacterium* when it is pre-cultured on a medium containing PGRs (Valvekens and Van Montagu 1988, Potrykus 1990, Sangwan *et al* 1992, Chateau *et al* 2000). Therefore, the morphogenetic potential of plant tissues needs to be controlled by altering the types and concentrations of carbon sources and PGRs inorder to create a robust regeneration system following transformation.

The objective of this study was to evaluate the effect of various carbohydrate sources and phytohormones and their appropriate concentrations on shoot induction and elongation rate to develop a conducive condition for growth and development and provide a better culture condition for soybean transformation using embryonic tip explant.

MATERIALS AND METHODS

Plant Materials

JS 335, JS 72-280, DS 228, and JS 72-44 soybean cultivars were taken for thestudy of carbohydrates on shoot induction, and JS 335 was used for the study of phytohormones at shoot elongation rate.

Seed Sterilization

Healthy and bold seeds were surface-sterilized by exposure to chlorine gas. The chlorine gas was prepared using a mixture of 5 mL HCl (39.6%) and 100 mL sodium hypochlorite (4%) and kept for 16-18 h (Liu and Wei 2002).

Explant Preparation

The sterilized seeds of taken cultivars were soaked in autoclaved distilled water overnight in the dark at 24°C. The embryonic tip explants were prepared by making a longitudinal cut along the hilum to separate the cotyledons and the seed coat was removed. The embryonic tip was then excised from the junctions of the hypocotyls and the primary leaves on the embryonic tip were removed to expose the meristem (Liu *et al* 2004).

Infection of Explants and Co-cultivation

Embryonic tip explants were immersed in *Agrobacterium tumefaciens* EHA105 suspension (0.7 OD600) harboring the binary vector pCAMBIA1305.1 for 10min, at room temperature. After inoculation, 10-15 explants (apical regions directed upwards) were placed in the sterile petri dish (90 mm) containing semi-solid co- cultivation media (CCM), which is composed of infection medium additionally supplemented with 0.06% agar-agar (Himedia, India), filter sterilized cysteine (200mg/l) and DTT (154.2 mg/l) with a piece of Whatman filter paper and then incubatedat 24°C in dark for 5-d.

Modified Culture Condition:

To see the Effect of Carbohydrates without Phytohormones

Transformed embryonic tip explants from CCM were transferred to shoot induction (SI) medium (1/10 Gamborg's B5 medium; B5 salts and vitamins, MSIII iron stock, 3% sucrose, 6 mM MES, 1.67 mg/l 6-BA, pH 5.7). Some modification wasdone in the SI medium to see the effect of carbohydrates on the shoot induction rate of transformed embryonic tip explants. For this study, SI media was supplemented with 3% (30 g/L) sucrose, maltose, or sorbitol (pH 5.6).

To see the Effect of Carbohydrates with Phytohormone

To confirm the effect of phytohormones on shoot induction rate, 0.4 mg/l IBAand 0.2 mg/l 6-BA were added in the medium with carbohydrate sources; sucrose, maltose, or sorbitol.

To see the effect of Phytohormone on Shoot Elongation Rate

Likewise, to see the effect of phytohormones on shoot elongation, the shoot elongation (SE) medium was supplemented with different concentrations of GA3 and IAA. We made 3 groups of IAA and GA3 where MS-B5 media was supplemented with 0.1 or 0.2 mg/l IAA in addition to either 0.5 mg/l (group I), 1 mg/l (group II) or

1.5 mg/l (group III) GA3 and compared the shoot elongation rate with control (Table 1). Phytohormones were added to the medium when the temperature dropped to 50- 60°C.

Statistical Analysis

Shoot induction and shoot elongation rate (%) in soybean cultivars was determined in three biological replications and each biological replication was measured in triplicate,the means and standard deviations were calculated. The data were statistically assessed using the one-way ANOVA. The comparison of the variation between means was performed via Fisher's least significant difference (LSD) value through DMRT at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Effect of different carbohydrate sources on shoot induction rate

To see the effect of different carbohydrate sources on shoot induction rate after transformation, SI media was supplemented with 30 g/L sucrose, maltose, or sorbitol.In this study, we observed that different carbohydrates influenced the frequency of plant regeneration. The highest frequency of shoot induction was observed when the transformed embryonic tips were cultured in the presence of maltose and gave an about2-fold increase in shoot induction than those cultured on a medium containing the same concentration of sucrose. Further study revealed +that shoot induction rate was observed high in media supplemented with 30 g/L maltose in all 4 cultivars JS335 (38.5%), DS 228 (50%), JS 72-280 (40%), and JS 72-44 (36%) as compared to sucrose which was 18.7%, 25%, 25%, and 20% in JS 335, JS72-280, DS 228, and JS 72-44, respectively (Fig. 1&2a; c). On the other hand, there was no shoot induction when SI media was supplemented with the same concentration of sorbitol (Fig 2e). Moreover,the optimum duration of shoot induction in media was 10-d. In this study, we concluded that it was favorable for the subculture of soybean embryonic tip explants to replace sucrose with maltose. Using maltose as a carbohydrate source instead of sucrose, not only promoted shoot induction as well helped in multiple and healthy shoot induction (Fig. 2c). The reason might be that sugar regulates the osmotic pressure of plant cells. Rahman et al. (2010) also assessed the effect of sucrose, glucose, and maltose for micropropagation of potato (*Solanum tuberosum*) and found maltose preferable over the other two in terms of multiplication rate like our study. Kumria et al. (2001) also suggested that the use of maltose had a more positive effect than sucrose in rice transformation. For regeneration, maltose was superior to sucrosein flax (Millam *et al* 1992) and triticale (Ainsley and Aryan 1998). We found no shoot induction in media containing sorbitol. Although, many previous studies suggested that sorbitol plays a positive role *in vitro* shoot development of the Rosaceae family (Ahmad *et al* 2007, Sotiropoulos *et al* 2006, Kadota *et al* 2001, Yaseen *et al* 2009).

Effect of phytohormone on shoot induction

Although the shoot induction rate was improved by replacing sucrose with maltose which was still low to get enough transformants. To find the improved shoot induction rate, transformed embryonic tips were sub-cultured onto 50 ml semi-solid SI medium containing 30 g/L maltose, sucrose, or sorbitol supplemented with 0.2 mg/l 6-BA and 0.4 mg/l IBA (pH 5.4) in a 100 ml flask. Statistical analysis of the data showed that significant differences were observed in the rate of shoot induction when 6-BA and IBA were added with different carbohydrate sources.

We found 75% shoot induction rate in JS 335 and 50% in DS 228, JS 72-280,and JS 72-44 after adding 6-BA, and IBA in SI medium containing sucrose which was almost 4-fold greater in JS 335, and 2-fold high in DS 228, JS 72-280 and JS 72-44 than when SI medium was supplied with sucrose solely (Fig. 1a & 2b).

A similar result was found in SI medium containing maltose, where we recorded83.4%, 62.5%, 56%, and 60% shoot induction frequency in JS 335, DS 228, JS 72- 280, and JS 72-44, respectively. The shoot induction rate was enhanced by 2.3, 1.2, 1.4, and 1.6-fold in JS 335, JS 72-280, DS 228, and JS 72-44, respectively after adding 6-BA, and IBA in media containing maltose (Fig. 1b & 2d). In contrast, we did not find any shoot induction in SI media containing sorbitol (Fig. 2f). Conclusively, the shoot induction rate was improved with the addition of phytohormones 6-BA and IBA.Moreover, the earliest and most vigorous multiple axillary shoots were also proliferated on a modified B5 medium containing 30 g/L maltose, 0.2 mg/l 6-BA, and 0.4 mg/l IBA. Thus, the augmentation of B5 medium with 6-BA and IBA along with maltose as a carbohydrate was found to be supportive for better shoot induction and vigorous shoot formation. Many studies also showed the effect of phytohormones of different concentrations in different crops. Kumari et al. (2015) found that MS medium (Murashige and Skoog 1962) supplemented with 2 mg/l 6-BA and 0.2 mg/l IAA was optimum for shoot regeneration in *Bacopa monniera*. In groundnut, a high regeneration efficiency was achieved by adding 66.6 µM 6-BA in the medium, while the highest number of shoot buds per explant was achieved by adding 20 µM 6-BA and 10 µM 2,4-D (Tiwari *et al* 2015). The highest regeneration efficiency for cotyledonary nodes of *Crambe abyssinica* was observed on a basic medium supplemented with 0.5 µM NAA and 2.2 µM 6-BA (Qi *et al* 2014).

Effect of phytohormone on shoot elongation of embryonic tip explant

After getting improved shoot induction, a healthy and good shoot length is required along with vigorous shoot formation for achieving healthy transformants. Induced shoots from embryonic tip explants of JS 335 were kept in various concentrations of IAA and GA3 and compared with the control. On the shoot elongation, two types of growth response were recorded; one is an average of shoot lengths, and the second is an average number of shoots. We recorded 3.4, 4.6, and 6.3 cm as the average shoot length and 2.6, 3.2, and 3.2 as the average shoot number in groups I, group II, and group III, respectively (Table 1). On the other hand, we observed only a single shoot with an average 2.1 cm shoot length in control. The result showed that increasing the concentration of GA3 with 0.1 or 0.2 mg/l IAA was found to be supportive not only for the better increase of shoot length but also for vigorous shoot formation (Fig. 3). In the present study, GA3 was found to be the stimulating agent for shoot elongation and IAA supported the GA3 for multiple shoot formation. In tea, the highest number of shoots per explant and shoot elongation rate was obtained using 3 mg/l 6-BA in combination with 0.5 mg/l GA3 during the shoot induction stage (Gonbad *et al* 2014). In Cerasus campanulate, MS medium enriched with 1 mg/l 6- BA, 0.1 mg/l IBA, and 2 mg/l GA3 significantly increased shoot elongation (Wang and Huang 2002). Moreover, 10 mg/l GA3 and 1 mg/l 6-BA could result in multiple plant formations with significantly taller shoots in sweet potatoes (20 mm) (Masekesa *et al* 2016). Li et al. (2017) also achieved the highest rate of shoot elongation (33.54%) when 1.0 mg/l GA3 and 0.1 mg/l IAA were added to the SE medium.

Table 1 Effect of different concentrations of IAA and GA3 on shoot elongation rate(%) in embryonic tip explants of JS 335 soybean cultivar

Category	IAA (mg/l)	GA3 (mg/l)	Avg. Shoot Length (cm)	Avg. no of shoots
Control	-	-	2.1 ± 1.1^{f}	1 ± 0.2^{d}
Group I	0.1	0.5	3.3 ± 0.3^{e}	2.5 ± 0.4^{c}
	0.2	0.5	3.6 ± 0.9^{d}	2.7 ± 0.5^{b}
Group II	0.1	1	4.8 ± 1.1^{b}	3.2 ± 1.4^{a}
	0.2	1	4.5 ± 0.5^{c}	3.3 ± 0.6^{a}
Group III	0.1	1.5	6.2 ± 1.2^{a}	3.2 ± 0.5^{a}
	0.2	1.5	6.4 ± 0.6^{a}	3.1 ± 0.7^{a}

* The results are expressed as mean ± standard deviation. Values followed with dissimilar letters differ significantly ($p<0.05$). Values with the same letter are not significantly different at $p<0.05$ level according to DMRT.

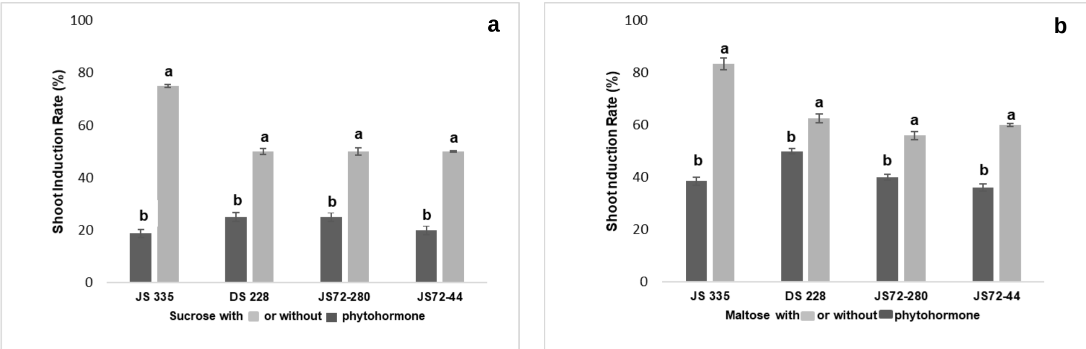

Fig. 1 Effect of (a) sucrose (with or without phytohormone) and (b) maltose on shootinduction rate in embryonic tips. *Values are means ± standard deviation. Values followed by a different letter are significantly different (*P<0.05*) in accordance with DMRT.

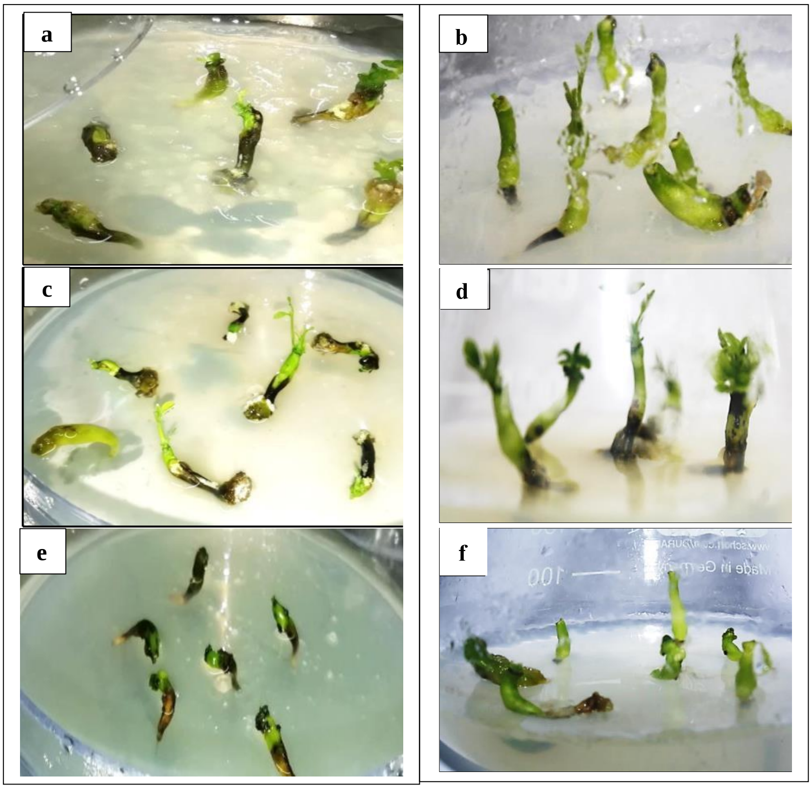

Fig. 2 JS 335 embryonic tip explants in shoot induction media after transformation containing (a) sucrose; (b) sucrose with phytohormone; (c) maltose; (d) maltose withphytohormones; (e) sorbitol; (f) sorbitol with phytohormone

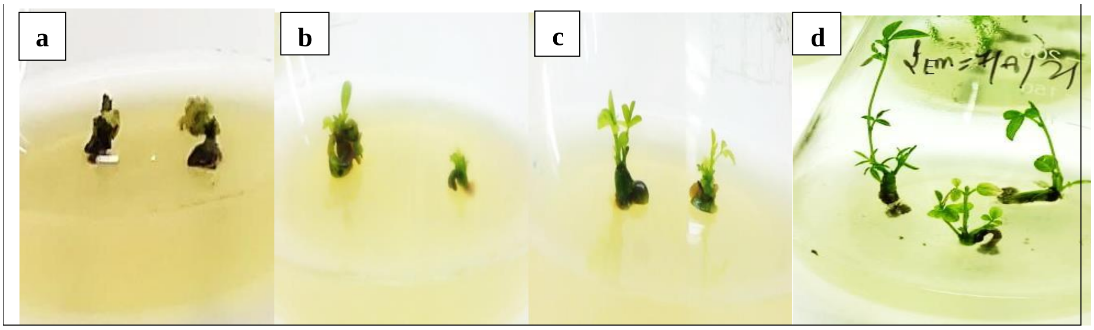

Fig. 3 JS 335 embryonic tip explants in Shoot Elongation media after transformation.(a) Control; (b) Group I (0.5 mg/ L GA3 + 0.2 mg/ L IAA); (c) Group II (1 mg/ L GA3+ 0.2 mg/ L IAA); (d) Group III (1.5 mg/ L GA3 + 0.2 mg/ L IAA)

CONCLUSION

By analysis, we get the conclusions that maltose had a significant effect on shoot induction followed by sucrose. Moreover, the inclusion of 6-BA and IBA in the SI medium containing sucrose or maltose showed an improved shoot induction rate (up to 83%). Therefore, the optimal carbohydrate source was found to be maltose for embryonic tips, and the optimal concentration of 6-BA and IAA was 0.2 mg/l and 0.4mg/l, respectively. The concentration of IAA and GA3 in the SE medium has significant effects on achieving better shoot length (average 6.3 cm) along with multiple (3.15 shoots per explant) shoot formation. The optimal concentration of GA3 and IAA for embryonic tips was 1.5 mg/l and 0.2 mg/l, respectively.

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Effectiveness of Social Media for the Dissemination of Soybean Production Technologies

B U DUPARE AND SAVITA KOLHE
ICAR-Indian Institute of Soybean Research, Khandwa Road, Indore
Email- soyextn@gmail.com
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ABSTRACT

The use of ICTs including social media tools of the recent introduction are becoming increasingly popular in agriculture especially among soybean stakeholders as is evident in the present study. The ICAR-Indian Institute of Soybean Research, an institution established for conducting basic and strategic research on soybeans has been promoting improved soybean production technologies using popular social media like Facebook page, YouTube channels, WhatsApp, Telegram groups, Instagram, and Twitter handles. After finalization of the content, the relevant video has been uploaded to its YouTube channel, and its link is shared on the remaining social platforms. The analytical data available on YouTube Studio revealed that the majority of the viewers (65%) belong to the age group ranging from 25-44 years. The majority of them prefer to watch videos related to the control of different insect pests and diseases. The viewers have shown their preference by watching videos on What to do and what not to do by placing them on the list of top popular videos. The institute's YouTube channel also hosts playlists and programs with innovative content like soya sanwad, an interactive session with progressive soybean growers and scientists, a virtual visit to the institute demonstration plot, and its news bulletin in the form of ICAR-Madhya Bharat Samachar, greatly been appreciated by the ICAR and its umbrella organizations across the country.

Keyword: Social media, Production technologies, Digital revolution

Dissemination of technologies and know-how is crucial for the development of agriculture in general and the farming community in particular. To achieve this goal, various methods and approaches were evolved, used, and refined in the past. The information/digital revolution being witnessed paved the way and avenues for efficient and effective technology delivery mechanisms for channeling the technical know-how to the millions of farmers in the quickest possible time. In today's digital world, life without mobile and internet is extinct. The Government of India has initiated various programs and issued guidelines that promote the use of digital media including social platforms for making information available about everything and/or anything at a single click. It has also launched a Unified Payment

Interface (UPI) and Single QR code making financial transactions even more convenient and easier for the common public. Information and Communication Technologies (ICTs) have occupied a greater significance in the technology delivery services in the present era. Earlier, television and radio have been major vehicles for disseminating agricultural information for a long time (Purushothaman *et al*., 2003) but the use of mobile, computing, and networking technologies provides new ways of technology transfer in an efficient way nowadays. Increased use of mobile subscriptions and its reach among the common people even among the rural people in the last decade have also increased the use of web-based services and applications like web portals and mobile apps.

World over, the popular social media used by the majority of the population include social networking sites (Facebook, LinkedIn, Myspace, etc.), video and photo sharing websites (Flickr, YouTube, Tumbler,Pinteres, etc.), blogs and microblogs (Blogger, Twitter, Instagram, etc.), forums, discussion boards and groups (Google groups, etc.), Wikis (Wikipedia, etc.), video and podcasts (Skype, etc.), video conferences and web conferences, Email and Instant Messaging (IM), BlackBerry Message (BBM), etc., socially integrated mobile text messaging (Line, WhatsApp, Viber, etc.), websites with social plug-ins and layers, social bookmarking (Delicious, Blink list, etc.), social news (Reddit, Propeller, Digg, etc.) and many more. Social media are forms of electronic communication through which users can create online communities for sharing information, ideas, personal messages, and other content (Merriam-Webster, 2013). Social media are a contemporary channel of digital communication that is composed of various evolving tools for discussion, interaction, and sharing of information among people. Simply stated, social media are digitally enabled platforms for communication through the internet in any form where the content is created and used by the users. Saravanan *et al.*, (2015) outlined many advantages of social media for its use in agricultural extension. The first and foremost is cost-effectiveness for reaching the very large clientele groups simultaneously. Secondly, it is location and client-specific and facilitates discussion on problem-oriented content. It can be easily accessed from mobile phones using internet facilities and promotes equality for the information users by making it accessible to all. Its use for technology dissemination brings all the actors/partners and stakeholders into a single platform. We can have readily available data about the promoted information in the form of the number of visitors, friends, followers, mentions, Facebook 'likes', conversation index, and number of shares, etc.

The results of a recent study carried out in Brazil (Collusi *et al.*, 2022) revealed social media platforms, such as WhatsApp, have grown increasingly important to farmers. Patel *et al.*, 2020 also conducted a study in Dewas district which showed that WhatsApp was able to increase the knowledge of the farmers on soybean production technology and farmers were at a high to low level of knowledge regarding social media-based technical information. Very recently, Dillen *et al.* 2023 compiled reviews of studies on the use of Social Media. Skaalsveen et al. (2020) found that farmers favored Twitter as a preferred means of social media as it enables easier peer interactions. Another study conducted by Das et al. (2019) observed that farmers use Facebook and Twitter to learn more about new technologies. A few studies also have pointed out that YouTube has enabled farmers to share videos of their practices as well as learn from other farmers, technology vendors, and experts (Burbi and Hartless Rose, 2016). WhatsApp has become popular with farmers creating or joining groups created by government or knowledge transfer bodies (Colussi et al., 2022; Vedeld et al., 2020).

Another study in the Indian context was also carried out recently by Manik Lal Roy *et al*. (2018) in which ICT tools used by the respondents were ranked on the basis of mean weightage score. It was found that mobile was in the first rank followed by television and radio. Internet was found to be in the last rank as far the usage is concerned. It was identified that factors viz. annual income, no. of crops cultivated, herd size, social participation, extension contact, mass media exposure, and innovativeness were highly associated with the extent of ICT usage at a 1% level of significance whereas factors viz. education, family size, landholding, training attended were associated with the extent of ICT usage at 5% level of significance. Nowadays, the outreach of smartphones even in rural India is increasingly becoming popular which enables farmers to assess the status of their crops or control their irrigation systems remotely have also been created (Mandi and Patnaik, 2019). The smartphone also gives farmers access to chat groups, newsletters, blogs, and bulletins that are meant for farmers to share information, experiences, and ideas.

Looking at the increased use of this social and digital platform among the farming community, the ICAR-Indian Institute of Soybean Research (ICAR-IISR) has initiated six social media platforms i.e. WhatsApp for farmers, Telegram channel for farmers and other stakeholders, Institute Facebook page, YouTube channel, Twitter handle, and Instagrams especially for the flow of information and also as feedback mechanisms related to the improved soybean production technologies developed by the Soybean R&D system. These platforms have enabled soybean growers of the major soybean states to exchange information on technologies/improved practices/ varieties and their technical know-how for putting these practices in the field condition. The present paper gives an overview of the performance social media platforms of ICAR-IISR and their effectiveness in dissemination of improved technologies among the farmers.

MATERIALS AND METHODS

In line with the popularity and use of WhatsApp groups, the ICAR-IISR launched its first social media in the year 2017 by creating a farmers' group on WhatsApp "IISR-Soy Farmer". Looking at the member ceilings and a very positive response received from extension personnel as well as progressive soybean growers the institute also launched another WhatsApp group "IISR-इंदौर-सोया साप्ताहिक सलाह "and telegram channel subsequently. Earlier, the ICAR-IISR was using its Facebook page created in the year 2017 simply to update the general public about institutional activities or breakthrough / research achievements of a general nature, and later on, the institute launched its YouTube Channel on 9th June 2020. The positive feedback and encouraging response received from the users, particularly the soy farmers and other stakeholders have motivated to initiate the institute's presence on Twitter as well as Instagram, gaining popularity in recent years. So, the institute has a presence on six social media (Fig,1) besides its Website and Android-based mobile app "Soybean Gyan" launched in the year 2017.

Fig. 1: Use of popular social media for transfer of technology among the soy stakeholders

The ICAR-IISR is using social media for dissemination of need-based and timely information like weekly advisories on soybean crops during the crop season, organization of various on and off-campus extension activities by the institute, etc. In order to keep track of the number of farmers/active users who follow the institute's social media, particularly the videos on various topics encompassing agronomic package of practices, management of biotic and abiotic factors like weeds, insect pests, and diseases, drought, etc., the videos are made available at single platform i.e. Institute YouTube Channel. The methodology followed includes conceptualization of information on relevant topics, videography, editing, and finalization in the form of a short video which is uploaded on the institute's YouTube Channel. After the video is uploaded on the YouTube channel, the link to these videos is immediately shared on remaining platforms like Facebook page, WhatsApp, Telegram, Twitter handle, and Instagram (in the form of reels).

The videos uploaded on the YouTube channel are categorized into different playlists like a package of practices, soybean varieties, insect management, disease management, weed management, research program, Weekly Advisory, *Soya Samvad* (an interactive session with farmers and scientists), AICRPS centers, IT/Commercialization, Live/ Demonstration programs and a news bulletin based program "ICAR-*Madhya Bharat Samachar*. Towards this, an effort is made to analyze these playlists/videos uploaded during the year 2022 on the institute's YouTube Channel. The present paper uses the data as available in the YouTube Studio/Analytica section to show the performance of the YouTube Channel and its content popularity among the viewers (both subscribers and non-subscribers).

RESULTS AND DISCUSSION

Social Media Initiatives of ICAR-IISR created for TOT on soybean: As has been mentioned earlier, the technical know-how on various issues including soybean varieties, agronomic package, and management of biotic and abiotic factors are synthesized in the form of Videos and are uploaded on the Institute's YouTube channel and thereafter link is shared on WhatsApp, Telegram, Twitter and Facebook page. Recently, the institute also started an Instagram channel that supports shorts/reels (60-second duration) and also uploaded on a YouTube channel.

A. Facebook Page

The Institute launched its Facebook Page in February 2017 (https://www.facebook.com/ICAR-Indian-Institute-of-Soybean-Research-Indore-507415769433553/) and is used on a regular basis is posting relevant useful information about events, technological recommendations, varietal release, etc. The page is very popular among soybean stakeholders. The likes and followers of the page have increased from 1950 to 2636 and 2245 to 3129 respectively. But consequent to the launch of Instagram, there is a decreasing trend (Fig 2) found in likes for the Facebook page. Since Facebook and Instagram have joint ownership, and videos/reels posted on either one are shared mutually on both these platforms. Therefore, the institute has recently launched an Instagram channel.

Fig 2. Screenshot of Facebook Page of IISR, Indore

B. YouTube Channel of ICAR-IISR

The institute is oerating its YouTube Channel since June 2020 for effective dissemination of soybean technologies. The YouTube Channel is accessible from the link https://www.youtube.com/channel/UCNdY5AsfPZqsCO8IxkAuSyQ/videos. There are more than 660 videos on different topics uploaded on the channel until now. The videos contain short and interactive discussions/advice by the institute scientists on various packages of practices, and the management of biotic and abiotic factors like weeds, insect-pest, and diseases. The comments on these videos are dealt with suitable responses by the experts. During the year 2022, a total of 126 videos were uploaded and were found viewed by 735,996 users (Fig. 3) making a 149% increase in total views and a 167% increase in watch time compared to 2021. The number of subscribers has also increased by 12,260 within a year considering the content coverage on only one crop i.e. soybean. It is heartening to know that the proportion of subscribers vs. non-subscribers is 59:41 which is a good trend indicating the popularity of the institute's YouTube channel and its content part (Fig. 4). It is also interesting to note the reach of our YouTube Channel crossing geographical boundaries as well as language reflecting its presence among the viewers of Turkey, Indonesia, Nepal, and Sweden besides Indians (Fig.5). The analytical data of the YouTube channel gives indication of how the viewers are making use of our videos. It is interesting to note that about 40% of the users watch our videos as suggested by someone, 35% through browsing, 8.1% by YouTube search, and 5.2% as external sources (Fig. 6). This may be due to the role played by other social media platforms of the ICAR-IISR namely WhatsApp/Telegram groups, Twitter and the users are sharing Facebook page through which the YouTube link of the videos.

Looking at the age profile of viewers (Fig. 7), it gives clear indication that the majority of the viewers are young having age group of 25-34 years (around 40%), followed by around 25% belonging to the age group of 35-44 years, 16% in the age group of 18-24 years. Only 19% of the YouTube Channel viewers belong to the age beyond 45 years. This may be due to the reason that young people are comfortable handling of social media profiles as well as familiarity and comfort of digital platforms. So far as the preferred content type of viewers is concerned (Fig. 8), 93% of them watch videos and the rest of them prefer to attend online activities like farm seminars, online discussions, and virtual meets.

The video content of the institute's YouTube channel is categorized into different playlists. The data in this regard are given in Fig. 9 which revealed that the majority of the viewers have shown interest in watching videos related to the control of different insect-pest and diseases causing yield losses (23%) followed by new and improved soybean varieties (17%), interactive session with progressive soybean growers (14%), agronomic practices (6%). The data also shows about 6% of viewers attended online programs like training/seminars involving farmer-scientist interaction organized by the ICAR-IISR from time to time. Fig. 10 presents an order of popular videos watched by the majority of the viewers during 2022.

According to the available data, majority of the viewers (12%) have watched a video on "What not to do, a list of common mistakes to be avoided by the soybean" followed by a video on control of girdle beetle, a major pest of soybean resulting in heavy yield losses (6%), guideline about the effectiveness of growth hormones in soybean (5%) Good agricultural practices (3%) and recent introduction of specialty and/or food grade soybean varieties (3%). The preferred list of watchable videos also includes videos on what/when/how to do in soybean, interactive sessions with progressive soya farmers, and so on.

CONCLUSIONS

The digital revolution of the recent past has paved the way for strengthening the process of technology dissemination through the effective and efficient use of ICT tools and social media in the agriculture sector. The increased use of ICT tools is offering a variety of solutions to the farming community on real real-time basis making the process of technology transfer more efficient, effective, trustworthy, and economically viable to the users. Looking at its enormous utilities more and more institutes, user groups, and agencies are now coming up to provide solutions to the farmers' issues through their mobile apps and ICT tools. The ICAR-IISR Indore is effectively using popular social media not only to provide technical support and guidance to soybean stakeholders but also to seek feedback on soybean technologies to provide solutions to them. Social media coupled with ICT tools could make a desirable impact among the farmers through the adoption of technologies and practices developed by the institute. Since ICTs has no boundaries, the information is also being accessed by the viewers of geographically scattered population. As is evident, the videos on the management of insect pest and diseases is a pertinent need of soybean growers covers as reflected in the YouTube data.

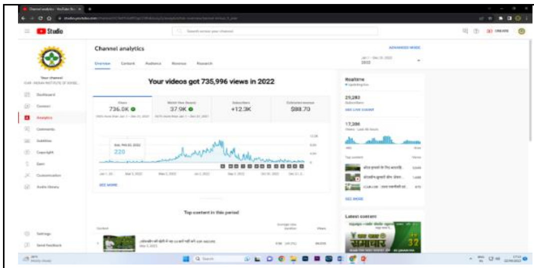

Fig.3: YouTube chanel data showing total views, watch time, subscribers

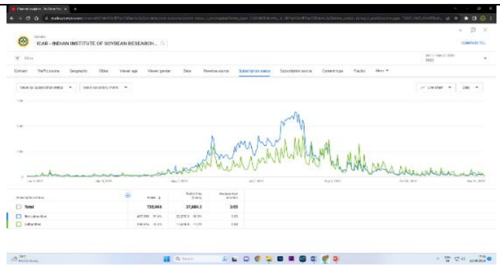

Fig. 4: Subscription status of viewers

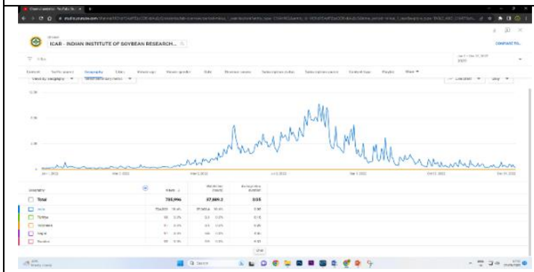

Fig. 5: Geographical distribution of viewers Channel

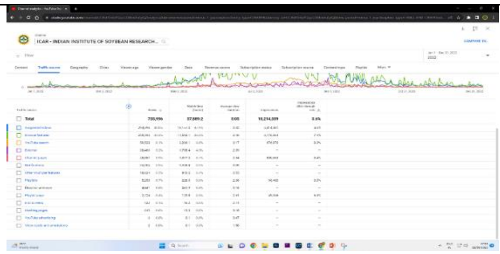

Fig.6: Traffic sources of how viewers knows about the videos uploaded on Institute YouTube Channel

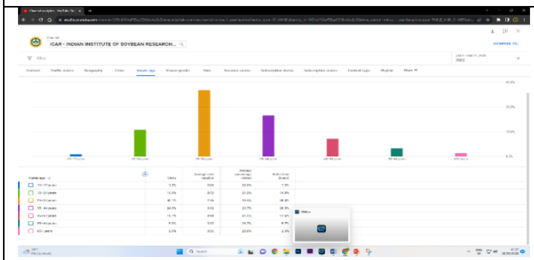

Fig.7: Age profile of YouTube Channel viewers

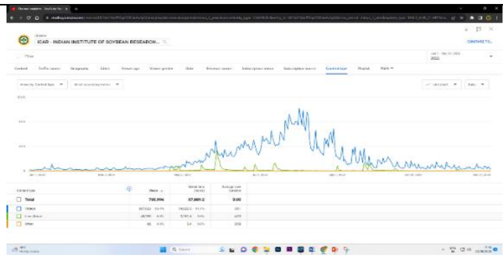

Fig. 8: Type of content preferred by the viewers

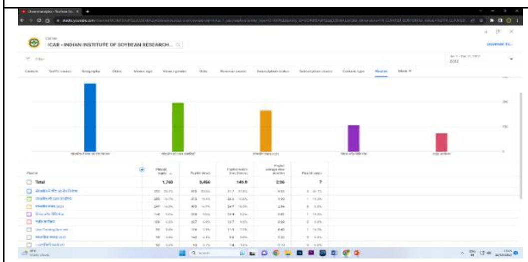

Fig.9: Playlist wise distribution of YouTube channel viewers

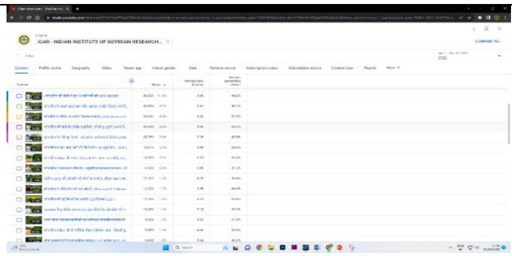

Fig.10: List of Top videos watched by the viewers

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Principle Component Analysis of Soybean genotypes in different growing Seasons

AKASH BARELA, MK SHRIVASTAVA, PAWAN K AMRATE, YOGENDRA SINGH, KUMAR JAI ANAND and SHIVANI JAWARKAR
Department of Plant Breeding & Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, (M.P.), India - 482004
Email: akashbarelagpb@gmail.com
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ABSTRACT

In present study, PCA was performed for economic traits of soybean. Out of twelve, only five principal components (PCs) exhibited more than 1.00 Eigen value and showed about 92.48% variability among the traits studied. First principal component recorded the highest variation 62.92% followed by 16.5% (second PC), 9.5% (third PC), 4.85% (fourth PC) and 2.63% (fifth PC). Total variation of five PCs was recorded as 62.92%. Rotated component matrix revealed that the PC1, which accounted for the highest variability was mostly related with traits such as number of pods per plant, number of seeds per plant, biological yield per plant and seed yield per plant. In second PC the traits viz., Days to maturity, days to 50% flowering were more related. The PC3 was dominated by Pod length (cm) and number of seeds per pod. The fourth component was more related to primary branches, plant height(cm), whereas PC5 was closely related to Harvest index (%) and 100 seed weight. On the basis of PCA most of the important yield and yield attributing traits were present in PC1 and PC2. The characters viz., plant height, number of pods per plant, number of pods per node, number of seeds per plant and number of seeds per pod are more important yield contributing traits, and putative genotypes identified as JS 22-15, JS 21-72, JS 22-53, NRC 137, JS 24-37, NRC 86, JS 22-44 and JS 23-05, on the basis of principal component analysis.

Keywords: Soybean, Eigen value, component matrix and principal component.

Soybean (*Glycine max* L. Merill) is known as 'golden bean' and miracle crop which is an efficient producer of two most scarce items in the world food economy i.e. high quality protein (40%) and oil (20%). It is a rich source of lysine (6.4%) and other essential amino acids, vitamins and minerals. It is also used as a raw material for the nutraceuticals, functional compounds such as isoflavones, tocopherol and lecithin and industrial products like paints, varnishes, adhesives and lubricants etc. It ranks first among oilseed crops in the world and India both. It contributes 25% of the total edible oil pool and 47% of the total oilseed production of the country. In the world scenario, United States of

America is the largest producer (around 50%) of soybean followed by Brazil, Argentina, China and India (Anonymous, 2014). In the past four decades, soybean has experienced a phenomenal growth rate of 15-20% per annum. In the year 2014-15, the soybean area reached to 10.02 m ha with total production 11.64 m t and the average productivity 1062 kg ha-1 in India. Madhya Pradesh is the largest contributor state occupying more than 50% area of the country followed by Maharashtra, Rajasthan, Karnataka and Andhra Pradesh. Madhya Pradesh is virtually known as synonym of "Soya State" as it has played a major role ever in the development and extension of soybean in all respect. The area of Madhya Pradesh has reached to the tune of 5.55 m ha with production of 6.02 m t with productivity of 1086 kg ha-1 Amrate *et al*, (2023) and Rahangdale *et al*, (2023a, b). Yield is determined by several traits therefore; a technique is required to identify and prioritize the important traits by minimizing the number of traits for effective selection and genetic gain. PCA, basically a well-known data reduction technique, identifies the minimum number of traits which contributes maximum variability and also ranks genotypes on the basis of PC scores.

MATERIALS AND METHODS

The present study was conducted under All India Coordinated Research Project on Soybean at Seed Breeding Farm, Department of Plant Breeding and Genetics, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidhyalaya Jabalpur (M.P.) to analyze Principal component analysis over different seasons and conclude pooled data (*Kharif* 2021, *Rabi* 2021-22 and *Kharif* 2022), in ninety genotypes of soybean. The field experiment was laid out in randomized complete block design (RCBD) with three replications. Ninety genotypes were planted with a spacing of 40 cm row to row and 7-8 cm plant to plant distance. All the recommended agronomical practices and plant protection measures were adopted to raise the healthy crop. The data was recorded on days to 50% flowering, days to maturity, plant height(cm), pod length (cm), primary branches, number of seeds per pod, number of pods per plant, number of seeds per plant 100 seed weight (g), biological yield, harvest index (%), seed yield per plant on a sample of 5 plants per replication in each genotype whereas for days to flowering and days to maturity data were taken on whole plot basis.

RESULT AND DISCUSSION

PCA provides a roadmap for how to reduce a complex data set to a lower dimension to sometimes hidden, simplified structures that often underlies it. Principal component analysis is appropriate for obtaining measures on a number of observed variables and to developing a smaller number of artificial variables (called principal component) that will account for most of the variance in the observed variables. The eigenvectors and the corresponding loadings for each individual from the PCA can be used as covariates within a logistic regression framework to account for the underlying structure. In present investigation, PCA was performed for quantitative traits of soybean are presented in table. Out of twelve, only five principal components (PCs) exhibited more than 1.00 Eigen value (table 1) and showed about 92.48% variability among the traits studied. First principal component recorded the highest variation 62.92% followed by 16.5% (second PC), 9.5% (third PC), 4.85% (fourth PC) and 2.63% (fifth PC). Total variation of five PCs was recorded as 92.48%.

Table 1: Eigen values, % variance and of promising genotypes of soybean over seasons

PC	Eigenvalue	% Vriance
1	220.276	62.92
2	57.9829	16.562
3	33.6044	9.5988
4	17.0113	4.8591
5	9.23678	2.6384
6	7.01398	2.0035
7	2.89874	0.828
8	1.29143	0.36889
9	0.439699	0.1256
10	0.207463	0.05926
11	0.071451	0.020409
12	0.055132	0.015748

Semi curve line obtained after fifth PC with little variation observed (fig.1) in each PC indicated that maximum variation was found in first PC, therefore selection for characters under first PC may be desirable. Rotated component matrix (tables 2 and 3) revealed that the PC1, which accounted for the highest variability was mostly related with traits such as number of pods per plant, number of seeds per plant, biological yield per plant and seed yield per plant. In second PC the traits viz., Days to maturity, days to 50% flowering were more related. The PC3 was dominated by Pod length (cm) and number of seeds per pod. Barela *et al*,(2022a, b). The fourth component was more related to primary branches, plant height(cm), whereas PC5 was closely related to Harvest index (%) and 100 seed weight. On the basis of PCA most of the important yield and yield attributing traits were present in PC1 and PC2.

Table 2: Interpretation of rotated matrix for the traits having values >0.5 in each PCs in pooled analysis

PC1	PC2	PC3	PC4	PC5
Seed yield per plant	Days to maturity	Pod length (cm)	Primary branches	Harvest index (%)
Number of seeds per plant	Days to 50% flowering	Number of seeds per pod	Plant height(cm)	100 seed weight (g)
Number of pods per plant	-	-	-	-
Biological yield	-	-	-	-

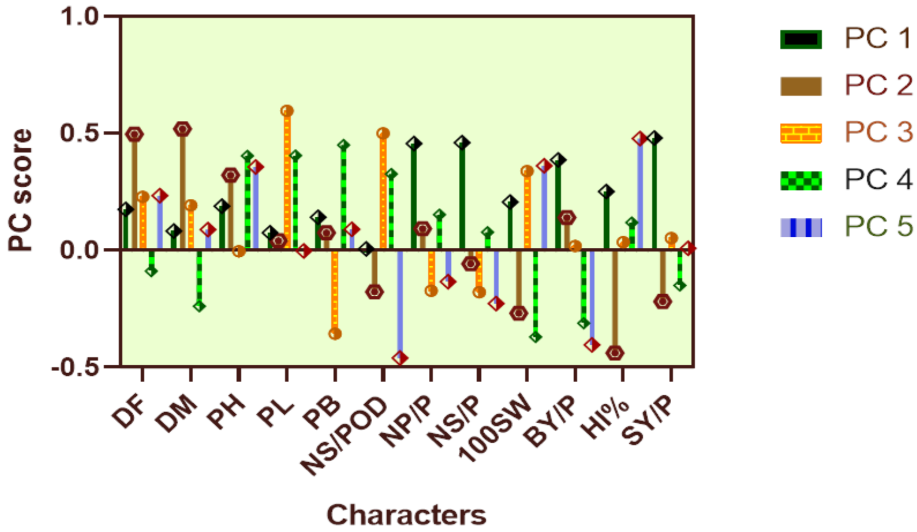
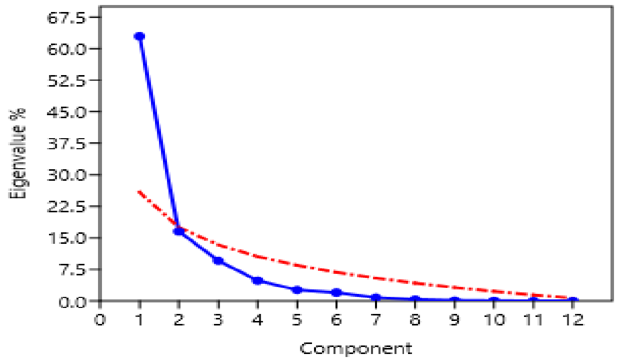


Figure 1. PCA rotated component matrix of major PCs

Table 3: PC values of rotation component matrix for twelve variables of ninety genotypes of soybean over seasons.

Characters	PC 1	PC 2	PC 3	PC 4	PC 5
Days to 50% flowering	0.028806	0.14054	-0.13949	0.2513	0.19601
Days to maturity	0.012588	0.23828	-0.40377	0.80142	-0.12609
Plant height(cm)	0.16431	0.91127	0.31103	-0.12277	-0.12786
Pod length (cm)	0.000826	0.009741	0.006847	0.004896	0.016805
Primary branches	0.011937	0.015733	0.00857	-0.02341	0.036827
Number of seeds per pod	0.000267	-0.0069	0.004755	-0.00179	-0.01275
Number of pods per plant	0.37091	0.11164	-0.13613	-0.1395	0.7932
Number of seeds per plant	0.88186	-0.18221	-0.10861	-0.04988	-0.39249
100 seed weight (g)	0.01339	-0.04183	0.075749	0.1495	0.25
Biological yield	0.1312	0.023356	-0.27724	0.033807	0.20314
Harvest index (%)	0.17259	-0.2059	0.77765	0.46826	0.14248
Seed yield per plant	0.097194	-0.04803	0.054491	0.11926	0.1423

Figure 2 & 3. Scree plot and biplot of different PC components.



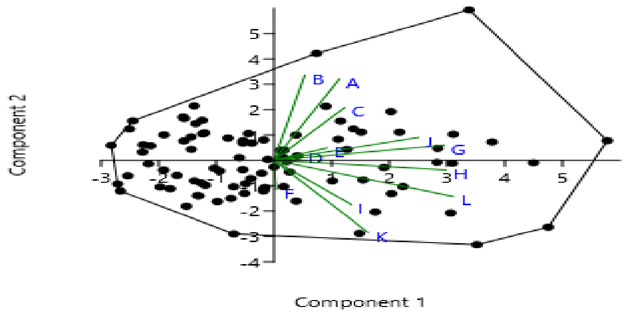


Table 4: Superior genotypes in different PCs

PCs	Superior genotypes in pooled analysis of 90 genotypes
PC1	JS 22-15, JS 21-72, JS 22-53, NRC 137, JS 24-37, NRC 86, JS 22-44, JS 23-05, NRC 125, JS 22-49, JS 22-17, JS 24-36, JS 22-31, JS 24-35, JS 20-116, AGS 163, JS 23-03, JS 22-42, JS 20-98, PS 1569, JS 22-46, JS 22-13, JS 20-105, JS 23-10, JSM 259
PC2	NRC 86, DCB 137, Cat 783, JS 22-32, JS 20-116, JS 22-14, JS 22-11, JS 22-30, JS 22-45, JS 20-105, JS 22-22, JS 22-46, JS 22-39, JS 20-98, JS 22-31, AVKS 206, JS 22-40, JS 22-41, JS 22-44, JS 22-50
PC3	JS 22-28, JS 23-03, JS 22-04, JS 22-31, JS 22-52, NRC 86, NRC 128, JS 22-25, NRC 130, JS 21-72, JS 22-54, JS 22-43, JS 22-48, JS 22-22, JS 22-32, PS 1092, JS 22-47, DS 3106, JS 22-41, JS 22-13
PC4	JS 22-04, JS 23-10, JS 22-20, JS 23-09, JS 22-35, JS 22-17, JS 22-25, JS 22-44, JS 21-72, JS 22-18, JS 22-45, JS 22-10, JS 22-14, AVKS 199, JS 22-39, JS 23-03, NRC 86, JS 24-35, JS 22-28, JS 20-98, JS 22-54
PC5	JS 23-05, JS 23-10, JS 22-52, JS 22-10, JS 22-19, JS 22-12, JS 22-46, JS 23-09, JS 22-53, JS 22-17, JS 22-42, JS 22-22, JS 23-08, JS 20-73, JS 22-51, JS 22-24

Similar studies of PCA in soybean genotypes by Uguru *et al*. (2012) screened seven genotypes of soybean under varying soil pH conditions. The PCA indicated that the first three principal components contributed 71.12 and 69.28 % of the total variability among the genotypes. Wang *et al*. (2013) performed PCA and showed cumulative contribution rates of the former four principal components to the variation of semi-determinate and determinate summer sowing soybean were 79.92% and 79.50%, respectively. Aondover *et al*. (2013) for pods/ plant, seed yield and plant height. Badkul *et al*. (2014) for vegetative phase, plant height, number of branches per plant and yield per plant. Hashash (2016) and Dubey (2018) observed principle components analysis showed that PC1 and PC2 having eigen values highest than unity explained 82.55% of total variability among soybean genotypes attributable to seed yield and accounted with values 67.77% and 14.78%, respectively. PC1 and PC2 noticed positive association with all and most genotypes, respectively and Jha *et al*. (2016) performed PCA for Identification and ranking of advanced genotypes of soybean based on combination of various phenotypic traits. Out of total principal components, five principal components were considered to be more important because they have more than one Eigen value that showed total variation among

CONCLUSION

The characters *viz.,* Seed yield per plant, number of seeds per plant, number of pods per plant and biological yield are more important yield contributing traits, and putative genotypes identified as JS 22-15, JS 21-72, JS 22-53, NRC 137, JS 24-37, NRC 86, JS 22-44, JS 23-05, NRC 125, JS 22-49, JS 22-17, JS 24-36, JS 22-31, JS 24-35, JS 20-116, AGS 163, JS 23-03, JS 22-42, JS 20-98, PS 1569, JS 22-46, JS 22-13, JS 20-105, JS 23-10 and JSM 259 on the basis of principal component analysis.

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Evaluation of Consortia of Microbial Formulations against Lepidopteran Foliage Pests of Soybean in Manipur

LALSANGZUALI C, NILIMA KARAM, H NANITA DEVI, T SUNANDA DEVI, L SOPHIA DEVI AND JETI KONSAM
College of Agriculture, Central Agricultural University, Imphal
E-mail: nilikaram@gmail.com
Received: 14/04/23; Accepted: 15/06/23

ABSTRACT

Efficacy of consortia of six commercial microbial formulations for the management of lepidopteran foliage pests of soybean crop viz., bihar hairy caterpillar (BHC) and bean leaf webber was undertaken as a field trial during kharif season of 2021-2022 at Central Agricultural University, Imphal, Manipur. Results of the study revealed that after three spray treatments, the differences in larval population of bihar hairy caterpillar were found significant over control treatment. After the spray treatment, the best combination of microbial formulation were Nomuraea rileyi + Bt commercial and Beauveria bassiana + Bt commercial harbouring only 2.89 BHC larvae/mrl each resulting in 87.2% and 80.4% reduction of BHC respectively. Similarly, mean larval population of bean leaf webber among different treatments also varied significantly. It could be observed that treatment with combination of Beauveria bassiana + Bt was the best harbouring only 0.67 larvae/mrl resulting in 79.88% reduction in population of leaf webber than preceding treatment. This treatment combination was similar in efficacy to Nomuraea rileyi + Bt commercial recording 0.78 larvae/mrl resulting in 71.94% reduction in population of leaf webber. Maximum seed yield was obtained with combination treatment of Beauveria bassiana + Bt giving the highest yield of 2537.04 kg/ha. The investigation proved that entomophagous fungi in combination with bacterial formulations is effective in managing lepidopteran foliage pests of soybean under field condition which can be used as a tool for eco-friendly pest control strategy.

Key words: Soybean, bihar hairy caterpillar, bean leaf webber, *Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Bt* Commercial

Soybean (*Glycine max* [L.] Merr.) is one of the most widely cultivated crops in the world. It is grown for its high-quality protein and oil, which have a variety of applications in agriculture and industry. Soybean is being cultivated in many North East states of India since time immemorial for the production of fermented and non-fermented foods. It is grown as a *kharif* crop on Manipur's hill slopes and terraces, foothills and valley soil. Soybean having a luxuriant growth with succulent leaves attracts a number of insect pests which are a nuisance element in soybean cultivation worldwide. Despite the wide variety of pests that attack soybean, the lepidopteran pests have long been the dominant taxa that reduce crop production. Bihar hairy caterpillar (BHC) and bean leaf webber are major foliage feeder insects attacking the crop primarily during vegetative stage under Manipur conditions (Karam *et al*, 2014). These pests attack soybean crop upto harvest reducing the crop yield (Singh and Singh, 1990). The use of chemical pesticides to control the pests has resulted in insect pest outbreaks, increased resistance to various insecticides, negative effects on natural enemies, risks to human and animal health and environmental contamination (Zadoks and Waibel, 1999). Scientists are speculating an increased outbreak of these pests as the increased resistance level against various insecticides had been reported. For these reasons, an effective alternative and an environmentally safe pest management strategy is the demand of the day and use of biocontrol agents viz., viruses, parasites, and predators is undoubtedly a boon to be harnessed (Parmar, 1993). However, utilizing entomopathogenic microbes in pest-control programme has a greater comparative cost, a narrower spectrum of insecticidal activity and lower persistence when compared to traditional chemical insecticides. The development of microbial pesticides based on more than a single microorganism is a proposed alternative to control mixed populations of insect pests as the efficacy of more than a single microorganism can give additive effect to control mixed population of insect pests when applied simultaneously. Keeping this in view, this study on effect of consortia of different microbial formulations against soybean lepidopteran foliage pests was undertaken for minimising the amount of pesticides used for pest control.

MATERIALS AND METHODS

The experiment was conducted with recommended soybean, variety JS 335 during *kharif* 2021-22 at CAU Research Farm, Andro, Imphal East. The plot size was 3.0 x 2.7 m with seeds sown at a row-to-row distance of 45 cm and 10 cm between plants and the crop was raised with standard agronomic practices. The experiment was laid out in Randomized Block Design with 7 treatments in three replications to compare the efficacy of microbial combinations against major lepidopteran foliage pests of soybean viz. bihar hairy caterpillar and bean leaf webber under Manipur conditions. Six consortia of commercial microbial formulations viz., combination of *Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi* and *Bacillus thuringiensis* (*Bt* Commercial) were tested along with control treatment. Three times spray application of microbial combination were given using knapsack sprayer; first at pest initiation stage at 40 days after sowing and another two at 20 days interval after the first treatment i.e., 2nd spray application at 60 days after sowing and 3rd spray at 80 days after sowing. Observations on the larval population of bihar hairy caterpillar and leaf webber were recorded per metre row length (mrl) leaving border rows one day before application (pre-treatment) and post treatment observations recorded on 3 and 7 days after spray treatment and mean population is computed. Seed yield in all the treatments was recorded at harvest and expressed as kg/ha. The field data on pest population obtained during the experiment were subjected to analysis of variance (ANOVA) after appropriate transformation for interpretation of results as per the procedure suggested by Gomez and Gomez (1993).

RESULTS AND DISCUSSION

Effect of microbial consortia on population of Bihar hairy caterpillar (BHC):

The data obtained from three spray treatment against larval population of BHC/mrl at pre-treatment, 3rd and 7th days after treatment were analysed (Table 1). High population of BHC prior to spray treatment in all plots varied from 14.00 – 36.67 larvae/mrl. After 3 days of spray application, reduction in population of BHC was observed ranging from 12.89 – 26.44 larvae/mrl however there was no significant differences in larval population due to treatment including untreated control. After 7 days of first spray treatment, larval population of BHC in soybean among different treatments recorded significantly superior results as compared to control treatment. Among the consortia of treatments, *Beauveria bassiana* + *Bt* commercial was observed with 5.22 larvae/mrl followed by *Nomuraea rileyi* + *Bt* commercial recording 8.00 larvae/mrl and both are statistically similar in effectiveness. Control treatment recorded maximum mean population of BHC i.e. 24.67 larvae/mrl. At the start of 2nd spray, there was a slight increase in the number of BHC larvae with population varying from 11.00 – 21.89 larvae/mrl. Same trend continued on 3 days after 2nd spray treatment with microbial formulations reducing population of larvae. There was a higher reduction in larval population on the 7th day after 2nd spray treatment with treatment combination of *Nomuraea rileyi* + *Bt* commercial and *Beauveria bassiana* + *Bt* commercial harbouring only 2.89 larvae/mrl each that resulted in 87.2% and 80.4% reduction of BHC respectively than the initial population recorded at the pest initiation stage just preceding treatment. Similar observations on larval mortality were made by Patil and Abhilash (2014) who reported that *Nomuraea rileyi* proved pathogenic to leaf eating caterpillars. Treatment consortia of only entomopathogenic fungal formulations viz., *Beauveria bassiana* + *Metarhizium anisopliae*, *Nomuraea rileyi* + *Metarhizium anisopliae* and *Beauveria bassiana* + *Nomuraea rileyi* recorded inferior results as compared to consortia between fungal and bacterial formulations. The present investigation is in partial agreement with Punwar and Sachan, 2005 who reported that entomopathogenic fungi are more effective in control of soybean leaf defoliators. No profound effect could be seen at commencement of 3rd spray treatment as incidence of BHC had lowered as the crop ages with control plots observed with less BHC population of 0.89 larvae/mrl. No population of BHC larva was observed after third spray treatment of microbial combination.

Effect of microbial consortia on population of bean leaf webber: Less significant population of bean leaf webber ranging from 2.89 – 3.67 larvae/mrl was observed in the experimental plots before application of microbial consortia (table 2). Non – significant reduction of leaf webber could be observed upto 3rd day after spray treatment. After 7th day of first spray treatment, all the microbial treatments recorded significantly superior result against larval population of bean leaf webber as compared to control treatment. General observations made during the study indicated that the fungal as well as bacterial insecticides even when combined take time to manifest infection on larvae of BHC and bean leaf webber as pathogenicity was initiated after 5-6 days after treatment gradually leading to increased larval mortality after a week of treatment. This corroborates the findings of Quesada-Moraga *et al.* (2006) that stated that efficiency of entomopathogenic microorganisms happens after 72 hours of treatment by penetrating the integument, trachea and the epithelial cells of target pests while damaging fat tissues and lethality may reached to 100%. At the start of 2nd treatment, larval population of bean leaf webber varies from 1.78 – 4.35 larvae/mrl. On the 7th day after 2nd spray treatment, treatment combination of *Beauveria bassiana* + *Bt* commercial was the most effective harbouring only 1.11 larvae/mrl each which was 66% reduced than initial population recorded at the pest initiation stage. Negligible count of leaf webber larvae could be observed by the 7th day of 3rd treatment which coincided with the decline of the pest naturally in the ecosystem as could be observed from control (1.11 larvae/m). However, it could be observed that all consortia of microbial formulations were significantly superior to control treatment with combination of *Beauveria bassiana* + *Bt* being the best with 0.67 larvae/mrl resulting in 79.88% reduction in population of leaf webber than what was observed before spray treatment. This treatment combination was similar in efficacy to *Nomuraea rileyi* + *Bt* commercial recording 0.78 larvae/mrl resulting in 71.94% reduction in population of leaf webber.

Yield of soybean: The yield of soybean increased significantly due to combination of microbial formulation as presented in table 3. The treatments produced 2333.33 kg/ha to 2537.04 kg/ha as compared to 1506.17 kg/ha in control treatment. Maximum seed yield was obtained with combination treatment of *Beauveria bassiana* + *Bt* giving the highest yield of 2537.04 kg/ha with an increase of 1030.87 kg/ha yield over control which was 40.6% higher than control. This treatment combination is at par with treatment combination of *Nomuraea rileyi* + *Bt* recording a yield of 2518.52 kg/ha with an increase of 1012.35 kg/ha yield over control which was 40.1 % higher than control. Similar yet partial agreement could be observed with the findings reported by Kamala Jayanthi and Padmavathamma (2001) that revealed sole application of *Bacillus thuringiensis* var. *kurstaki* resulted in 81 % higher yield than the control.

CONCLUSION

Treatment consortia of *Beauveria bassiana* + *Bt* and *Nomuraea rileyi* + *Bt* proved promising in lowering population of lepidopteran foliage pests of soybean and also recorded higher seed yield as compared to control treatment. The present study is in agreement with Lacey *et al*. (2015) who stated that the efficacy of more than a single microorganism gives additive effect to control mixed population of insect pests when applied simultaneously. Combination of different microbial formulations takes advantage of the particular strength of each agent minimizing their biological limitations, lowering the dose requirement and improving the speed of kill.

Table 1. Effect of microbial consortia on population of Bihar hairy caterpillar/mrl in soybean

	Treatments	PT	1st spray 3DAT	1st spray 7DAT	PT	2nd spray 3DAT	2nd spray 7DAT	PT	3rd spray 3DAT	3rd spray 7DAT
T1	*Beauveria bassiana* (2 kg/ha) + *Metarhizium anisopliae* (2 kg/ha)	36.67 -6.08	26.44 -5.13	16.44 -4.1	18.11 -4.31	12.89 -3.65	6.67 -2.66	0.22 -0.83	0	0
T2	*Nomuraea rileyi* (2 kg/ha) + *Bt* (1 kg/ha)	22.67 -4.72	20.44 -4.5	8 -2.89	11.11 -3.37	6.89 -2.69	2.89 -1.7	0 -0.71	0	0
T3	*Nomuraea rileyi* (2 kg/ha) + *Metarhizium anisopliae* (2 kg/ha)	19.33 -4.29	16.89 -4.15	13.11 -3.63	12.44 -3.55	9.78 -3.17	3.33 -1.94	0.33 -0.9	0	0
T4	*Beauveria bassiana* (2 kg/ha) + *Nomuraea rileyi* (2 kg/ha)	14 -3.74	13.56 -3.74	11.56 -3.45	14.44 -3.85	11.78 -3.49	6.67 -2.66	1 -1.21	0	0
T5	*Beauveria bassiana* (2 kg/ha) + *Bt* (1 kg/ha)	14.78 -3.82	12.89 -3.57	5.22 -2.39	11 -3.36	7.44 -2.75	2.89 -1.81	0.22 -0.83	0	0
T6	*Metarhizium anisopliae* (2 kg/ha) + *Bt* (1 kg/ha)	23.56 -4.84	15.33 -3.89	14.22 -3.83	12.67 -3.53	10 -3.15	4.89 -2.29	0.44 -0.92	0	0
T7	Untreated control	24.22 -4.91	22.44 -4.74	24.67 -4.95	21.89 -4.72	22.89 -4.83	13.89 -3.79	0.89 -1.06	0	0
	SE ±	-	-	0.5	0.33	0.27	0.32	-	-	-
	CD @ 5%	NS	NS	1.1	0.73	0.6	0.69	NS	-	-

Figures in parentheses are square root (x + 0.5) values

Table 2. Effect of microbial consortia on population of bean leaf webber/mrl in soybean

Treatments		1st spray			2nd spray			3rd spray		
		PT	3DAT	7DAT	PT	3DAT	7DAT	PT	3DAT	7DAT
T1	*Beauveria bassiana* (2 kg/ha) + *Metarhizium anisopliae* (2 kg/ha)	2.89	2.89	1.44	1.78	1.56	1.33	1.44	1.22	1.11
		-1.83	-1.84	-1.39	-1.51	-1.43	-1.35	-1.38	-1.3	-1.26
T2	*Nomuraea rileyi* (2 kg/ha) + *Bt* (1 kg/ha)	2.78	2.44	1.67	2	1.44	1.44	1.33	1.11	0.78
		-1.81	-1.7	-1.46	-1.57	-1.39	-1.39	-1.35	-1.26	-1.13
T3	*Nomuraea rileyi* (2 kg/ha) + *Metarhizium anisopliae* (2 kg/ha)	3	2.67	2.11	2.44	1.89	1.78	1.67	1.33	1.22
		-1.87	-1.77	-1.61	-1.71	-1.54	-1.51	-1.47	-1.34	-1.31
T4	*Beauveria bassiana* (2 kg/ha) + *Nomuraea rileyi* (2 kg/ha)	3.11	2.56	1.89	2.44	1.78	1.67	1.56	1.22	0.89
		-1.9	-1.72	-1.54	-1.72	-1.5	-1.46	-1.4	-1.3	-1.16
T5	*Beauveria bassiana* (2 kg/ha) + *Bt* (1 kg/ha)	3.33	2.67	1.33	1.78	1.33	1.11	0.78	0.67	0.56
		-1.94	-1.75	-1.34	-1.5	-1.34	-1.26	-1.12	-1.07	-1.02
T6	*Metarhizium anisopliae* (2 kg/ha) + *Bt* (1 kg/ha)	3.67	2.78	2.44	3	2	1.78	1.44	0.89	0.78
		-2.04	-1.81	-1.71	-1.86	-1.57	-1.51	-1.39	-1.18	-1.13
T7	Untreated control	3.22	3.89	4.33	4.33	4.56	3.78	3	2.44	1.11
		-1.92	-2.07	-2.2	-2.18	-2.24	-2.06	-1.86	-1.71	-1.27
	SE ±	-	-	0.17	0.17	0.14	0.14	-	0.15	0.1
	CD @ 5%	NS	NS	0.37	0.36	0.31	0.3	NS	0.34	0.2

Figures in parentheses are square root (x + 0.5) values

Table 3. Effect of microbial consortia on yield of soybean (var. JS – 335)

	Treatments	Yield (kg/ha)
T1	*Beauveria bassiana* (2 kg/ha) + *Metarhizium anisopliae* (2 kg/ha)	2358.02
T2	*Nomuraea rileyi* (2 kg/ha) + *Bt* (1 kg/ha)	2518.52
T3	*Nomuraea rileyi* (2 kg/ha) + *Metarhizium anisopliae* (2 kg/ha)	2358.02
T4	*Beauveria bassiana* (2 kg/ha) + *Nomuraea rileyi* (2 kg/ha)	2370.37
T5	*Beauveria bassiana* (2 kg/ha) + *Bt* (1 kg/ha)	2537.04
T6	*Metarhizium anisopliae* (2 kg/ha) + *Bt* (1 kg/ha)	2333.33
T7	Untreated control	1506.17
SE ±		70.52
CD @ 5%		153.64

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In -Vitro Evaluation of Fungicides Against *Colletotrichum Truncatum* Causing Anthracnose of Soybean

SALONI MANDLOI [1,2], SAPNA JAISWAL[1,2], LAXMAN SINGH RAJPUT[2,3], SANJEEV KUMAR[2], VENNAMPALLY NATARAJ[2], SHIVAKUMAR M[2], HEMANT SINGH MAHESHWARI[2], RAJNEESH SHARMA[3], VIBHA PANDEY[1], JAYANT BHATT[1]*

[1]Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, 482 004, Madhya Pradesh, India, [2]ICAR-Indian Institute of Soybean Research, Indore, 452 001, Madhya Pradesh, India, [3] ICAR-Central Arid Zone Research Institute, Jodhpur, 342 003, Rajasthan, India

Email: - laxman0742@gmail.com

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ABSTRACT

Soybean anthracnose, a widespread and significant disease affecting soybean cultivation worldwide, leads to substantial losses in both crop yield and quality. To evaluate its impact, ten fungicides were subjected to testing using the poison food technique against Colletotrichum truncatum. These fungicides, namely Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w FS, Carboxin 37.5% + Thiram 37.5% WS, Carbendazim 25% + Mancozeb 50% WS, Picoxystrobin 22.52% w/w SC, Tebuconazole 25.9% EC, Hexaconazole 5% EC, Kresoxim-methyl 44.3% SC, Tebuconazole 10% + Sulphur 65% WG, Pyraclostrobin 20% WG, and Propiconazole 25% EC, were tested at four different concentrations: 100 ppm, 250 ppm, 500 ppm, and 1000 ppm. The outcome revealed that Propiconazole 25% EC and Carboxin 37.5% + Thiram 37.5% WS fungicides exhibited the highest level of inhibition against mycelium growth.

Keywords: Fungicides, Anthracnose, Antibiotic Assay

Soybean (*Glycine max* (L.) Merrill) is a valuable and oilseed crops that produces about two-thirds of the world's protein and one fourth of the world's edible oil (Singh *et al*., 2021a, Nataraj *et al*., 2019). In order to share total output, the largest soybean producing countries include USA, Brazil, Argentina, China and India (Gupta *et al*. 2012). India ranks fifth in soybean output, with 10.45 million metric tons produced over an area of 12.7 million hectares (USDA 2021). Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Andhra Pradesh, and Chhattisgarh are the biggest soybean producing states. The state of Madhya Pradesh has been designated as a "soybean state". The search for greater soybean yields is hampered by biotic and abiotic stressors (Rajput *et al*., 2021; Singh *et al*., 2021b). A wide range of pathogens, including fungus, bacteria, viruses, and nematodes, can cause crippling diseases in soybean, making it especially susceptible to them. Approximately 12–20 percent of the entire harvest loss is attributed to these diseases, which have a substantial negative impact on soybean output (Mittal *et al*., 2001). It is now understood that the disease known as soybean anthracnose, which is caused by the pathogen *Colletotrichum truncatum* (Schw.) Andrus and Moore, has a complex aetiology. Worldwide humid tropics and tropical climates are affected by its economic effects (Boufleur *et al.,* 2021b). An estimated 1.17 million tonnes of output were lost to anthracnose in India alone in 2006 (Subedi *et al*., 2015). In India, soybean anthracnose caused 16-25% of yield loss (Nataraj *et al*., 2020). Recently, Rajput *et al*. (2022) revealed that 1% increase in soybean anthracnose disease severity leaded to reduced 115 kg/ha soybean grain yield. The gradual spread of the infection includes all stages of soybean growth, from flowering through pod maturation. Pre-emergence damping-off may cause infected seeds sown in later planting seasons to succumb, which would reduce plant populations. Infection can also spread from cotyledons to young stems, encouraging the development of cankers and an early plant death (Rajput *et al*., 2021; Nataraj *et al*., 2023).The disease has characteristic signs, such as the formation of brown patches with a central grey area on the upper leaves and burnt-looking lower leaves. Long-term exposure to humidity levels that are persistently high, up to 90%, can aggravate the illness and cause other symptoms such necrosis of leaf veins, leaf rolling, petiole canker, and premature defoliation (Rajput *et al*., 2021; Nataraj *et al*., 2023). The pod blight phase, which is extremely harmful, is of special concern. This phase is initially distinguished by the emergence of amorphous brown patches on stems, pods, and petioles ((Rajput *et al*., 2021; Nataraj *et al*., 2023).

In central India, the heavy and continuous rain at pod filling leads to develop soybean anthracnose symptom vigorously. Most of soybean producing state faces huge challenge of soybean anthracnose due to none available of resistant commercial cultivar (Rajput *et al*., 2021; Nataraj *et al*., 2023). Recently, Kumar *et al*., (2023) identified milkweed (*Euphorbia geniculata*) as alternative source for soybean anthracnose that act as primary source for soybean anthracnose. Therefore, 10 fungicides have been employed to combat this disease, including Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w FS, Carboxin 37.5% + Thiram 37.5% WS, Carbendazim 25% + Mancozeb 50% WS, Picoxystrobin 22.52% w/w SC, Tebuconazole 25.9% EC, Hexaconazole 5% EC, Kresoxim-methyl 44.3% SC, Tebuconazole 10% + Sulphur 65% WG, Pyraclostrobin 20% WG, and Propiconazole 25% EC.

MATERIAL AND METHODS

The present investigation was carried out at Division of Crop Protection, ICAR- Indian Institute of Soybean Research, Indore, Madhya Pradesh, India during the year, 2023.

Isolation of the pathogen

Soybean plant leaves and petioles exhibiting distinct symptoms, such as brown spots with a gray center on upper leaves and a burnt appearance on lower leaves, were collected from ICAR-IISR, Indore in 2022. The infected portions were carefully cut into 5 to 6 mm pieces and subjected to surface sterilization using a 1.0% sodium hypochlorite solution for one minute. After three washes with double distilled sterilized water, the sterilized bits were transferred to Petri plates containing sterile PDA medium under aseptic conditions. These plates were then incubated at a constant temperature of 26 ± 1 °C for four days to promote robust fungal growth. The isolated fungi were purified using the hyphal tip technique, and their growth characteristics, including morphology and cultural formation, were utilized for identification. Based on the observed traits, the fungi were identified as *Colletotrichum truncatum*. The pure fungal culture was sub-cultured on PDA slants and maintained in the laboratory at 27 ± 2°C for 15 days. Additionally, mother culture slants were preserved in a refrigerator at 5°C. Regular sub-culturing, once a month, was performed to maintain the cultures for future research purposes.

Efficacy of fungicides and antibiotic assay

In the pursuit of assessing the inhibition of mycelial growth, an *in-vitro* study was carried out employing the poisoned food technique on PDA (Potato Dextrose Agar) medium (Rajput *et al*., 2016). The experiment adhered to a Completely Randomized Design (CRD) structure, encompassing 10 distinct treatments, each subjected to three replications. The study involved the evaluation of ten systemic fungicides: Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w FS, Carboxin 37.5% + Thiram 37.5% WS, Carbendazim 25% + Mancozeb 50% WS, Picoxystrobin 22.52% w/w SC, Tebuconazole 25.9% EC, Hexaconazole 5% EC, Kresoxim-methyl 44.3% SC, Tebuconazole 10% + Sulphur 65% WG, Pyraclostrobin 20% WG, and Propiconazole 25% EC. These fungicides were tested at four different concentrations: 100 PPM, 250 PPM, 500 PPM, and 1000 ppm. Fungicide suspensions were meticulously prepared in PDA by incorporating the appropriate quantity of each fungicide to achieve the desired concentration, based on the active ingredient content. Sterilized Petri plates were subsequently filled with this "poisoned" medium. Mycelial discs, measuring 4 mm in diameter, were carefully extracted from the periphery of four-day-old fungal cultures and positioned at the center of the plates. The plates were then incubated at a temperature of 28 ± 2°C until the fungal growth reached the periphery of the control plate. For each treatment, three replications were consistently maintained. The average colony diameter was measured in two directions, and the inhibition percentage was computed (Rajput *et al.,* 2016).

$$I = 100(C-T) / C$$

Where, I = Per cent inhibition of mycelium growth;
C = Growth of mycelium in control;
T = Growth of mycelium in treatment.

RESULT

The results, as presented in Tables 1 and 2, unequivocally indicate that all the fungicides subjected to testing significantly impeded the mycelial growth of the fungus. Among the ten systemic fungicides initially tested after 7 days of incubation, Propiconazole 25% EC (90.00 %), Carboxin 37.5% + Thiram 37.5% WS (90.00%), and Carbendazim 25% + Mancozeb 50% WS (90.00%) demonstrated the highest mean mycelial inhibition percentage, surpassing significantly all other fungicide. Among the different concentration of fungicides, 1000 ppm provide significant higher mean mycelia inhibition compared to other concentration. Following closely were Tebuconazole 25.9% EC (57.08%) and Pyraclostrobin 20% WG (46.94%). Notably, Propiconazole 25% EC, Carboxin 37.5% + Thiram 37.5% WS, and Carbendazim 25% + Mancozeb 50% WS maintained their superior inhibitory effects across concentrations of 100 ppm, 200 ppm, 500 ppm, and 1000 ppm, showcasing a significant difference compared to other treatments. In the subsequent reading (10 days after incubation), Propiconazole 25% EC (90.00%) and Carboxin 37.5% + Thiram 37.5% WS (90.00%) again displayed maximum mean mycelial inhibition, surpassing significantly all other treatments. Among the different concentration of fungicides, 1000 ppm provided significant higher mean mycelia inhibition compared to other concentration. Similar to the first reading, at concentrations of 100 ppm, 200 ppm, 500 ppm, and 1000 ppm, Propiconazole 25% EC and Carboxin 37.5% + Thiram 37.5% WS maintained their significant inhibitory effects, distinguishing them from other treatments. Among the fungicides tested, namely Propiconazole 25% EC and Carboxin 37.5% + Thiram 37.5% WS, exhibited noteworthy mycelial inhibition across their tested concentrations and emerged as highly effective against *Colletotrichum truncatum* under in-vitro conditions.

DISCUSSION

Based on the results presented in Tables 1 and 2, it is evident that the tested fungicides significantly hindered the mycelial growth of *Colletotrichum truncatum*.

Particularly, Propiconazole 25% EC, Carboxin 37.5% + Thiram 37.5% WS, and Carbendazim 25% + Mancozeb 50% WS demonstrated the highest mean mycelial inhibition percentage after 7 days of incubation, showcasing their efficacy in impeding the growth of the fungus. Moreover, these fungicides maintained their inhibitory effects across varying concentrations (100 ppm, 200 ppm, 500 ppm, and 1000 ppm), further underlining their potency against *Colletotrichum truncatum*. Notably, 1000 ppm concentration consistently exhibited higher mycelial inhibition compared to other concentrations. In the subsequent reading (10 days after incubation), Propiconazole 25% EC and Carboxin 37.5% + Thiram 37.5% WS continued to exhibit maximum mean mycelial inhibition, emphasizing their persistent and effective control over the growth of the pathogen. This consistency across concentrations substantiates their potential as reliable agents for managing *Colletotrichum truncatum* under *in-vitro* conditions. In recent studies in India and world, researchers have emphasized the importance of effective fungicides in managing fungal diseases in crops. Propiconazole has been recognized for its potent antifungal properties and effectiveness against a range of plant pathogens (Xing *et al*., 2019, Xu *et al*., 2021). Additionally, Carboxin and Thiram have shown significant antifungal activities against various fungal pathogens, including those affecting crops (Bulat *et al*., 2019). These findings align with the current study, emphasizing the efficacy of Propiconazole and Carboxin + Thiram combination in inhibiting *Colletotrichum truncatum* growth.

In conclusion, Propiconazole 25% EC and Carboxin 37.5% + Thiram 37.5% WS have demonstrated notable inhibitory effects on the mycelial growth of *Colletotrichum truncatum* across varying concentrations, making them promising candidates for the effective control of this pathogen. Further studies, including *in-vivo* trials and field evaluations, are warranted to validate these findings and assess the practical efficacy of these fungicides in the management of *Colletotrichum truncatum* in real-world agricultural settings.

Table-1 ***In-vitro*** **evaluation of after 7th days reading of combination product fungicides against** ***Colletotrichum truncatum***

Fungicide	Per cent inhibition of mycelial growth				Mean
	Concentration				
	100 ppm	250 ppm	500 ppm	1000 ppm	
Penflufen 13.28% + Trifloxystrobin 13.28%	25.62^{h-k}	39.12^{c-h}	37.71^{c-i}	36.43^{d-i}	34.72^{d}
Picoxystrobin 22.52%	17.34^{kl}	21.00^{j-l}	45.00^{b-e}	48.83^{b-d}	33.04^{d}
Tebuconazole25.9%	37.26^{d-i}	45.63^{b-e}	55.43^{b}	90.00^{a}	57.08^{b}
Hexaconazole 5%	10.64^{l}	24.79^{i-k}	25.69^{h-k}	26.51^{h-k}	21.91^{e}
Kresoxim-methyl 44.3%	27.28^{g-j}	30.35^{f-k}	40.50^{c-g}	40.49^{c-g}	34.66^{d}
Tebuconazole 10% + Sulphur 65%	24.79^{i-k}	25.69^{h-k}	35.24^{d-i}	32.45^{e-j}	29.54^{d}
Carboxin37.5% + Thiram 37.5%	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}
Carbendazim 25%+ Mancozeb 50%	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}
Pyraclostrobin 20%	37.39^{d-i}	43.69^{b-f}	51.20^{bc}	55.49^{b}	46.94^{c}
Propiconazole 25%	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}
Mean	45.03^{d}	50.03^{c}	56.07^{b}	60.02^{a}	

Table-2 ***In-vitro*** **evaluation of after 10th days reading of combination product fungicides against** ***Colletotrichum truncatum***

Fungicide	Per cent inhibition of mycelial growth				Mean
	Concentration				
	100 ppm	**250 ppm**	**500 ppm**	**1000 ppm**	
Penflufen 13.28% + Trifloxystrobin 13.28%	33.94g-i	41.80c-f	39.20e-h	36.80e-h	37.93f
Picoxystrobin 22.52%	12.12j	15.75j	42.12c-f	52.73b	30.68g
Tebuconazole25.9%	40.20d-g	47.24b-d	52.75b	90.00a	57.54c
Hexaconazole 5%	29.17i	29.58i	32.15hi	35.76f-i	31.66g
Kresoxim-methyl 44.3%	42.76c-f	41.16d-g	41.16d-g	40.83d-g	41.48e
Tebuconazole 10% + Sulphur 65%	42.76c-f	38.86e-h	42.44c-f	44.04c-e	42.02e
Carboxin37.5% + Thiram 37.5%	90.00a	90.00a	90.00a	90.00a	90.00a
Carbendazim 25%+ Mancozeb 50%	48.84bc	51.43b	90.00a	90.00a	70.06b
Pyraclostrobin 20%	42.44c-f	47.25b-d	42.03c-f	51.63b	45.84d
Propiconazole 25%	90.00a	90.00a	90.00a	90.00a	90.00a
Mean	47.22d	49.30c	56.18b	62.18a	

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Decadal cropping pattern and expansion dynamics of Soybean in Madhya Pradesh, India

RAM MANOHAR PATEL[1], P SHARMA[2], K BUNKAR[3], A RAMESH[1], N KHANDEKAR[1]*

[1]ICAR - Indian Institute of Soybean Research, Khandwa Road, Indore – 452001, [2]ICAR - National Institute of Agricultural Economics and Policy Research, Pusa, New Delhi-110 012, [3]Institute of Computer Science, Vikram University, Ujjain- 456010

E-mail: nitakhandekar@yahoo.co.in

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ABSTRACT

Soybean is a major oilseed crop of India, commercially grown since 1970s and presently cultivated between 15 °N to 25 °N longitude. It's area is constantly increasing over the last fifty years. Farmers initially had replaced previously fallow lands to soybean cultivation in Madhya Pradesh. Later, it replaced less remunerative and less adaptive crops, the area however, fluctuates due to the climatic variance and volatile market. The study aims to investigate the decadal growth and changes in cropping pattern of the crop. It was assessed by adopting Markov chain analysis to know the changes in area from one crop to another through transitional probability matrices. Major rainfed crops competing with soybean in Madhya Pradesh have been selected for the analysis. Since the 1970s, soybean area has increased by about 800 times (from 7.7 to 6194 thousand hectares), sorghum lost 95% (from 2155 to 108 thousand hectares) of the area to other crops (groundnut, finger millets, other oilseeds, pigeon pea and other pulses etc.) but not to soybean directly; current fallow and other minor crops area had declined by about 50% with about 12, -5.5, -1.4 and -1.9 percent CAGR, respectively. The transition probability matrices (TPM) revealed that area under soybean has declined in its area share as against previous year, from 100 percent (in 1970-79) to 33 percent (in 2010-19) over the years and becoming unstable. Data reveals, it gained area mainly from current fallows, maize, other minor crops, and cotton. In conclusion, soybean has lost its small share of the previous year area after the initial period mainly to maize and other minor crops and the growth rate of soybean has declined in the recent decade. Therefore, to minimize import dependency of oils and to be self-sufficient, suitable promotional policies have to be envisaged to motivate farmers to cultivate soybean on the current fallow lands not only in Madhya Pradesh but also all over the country.

Keywords: Soybean, Current Fallow, Sorghum, Growth Rate, Markov chain, Transition Probability Matrix

Agriculture contribution to national gross domestic product (GDP) is around 19.9 percent in 2020-21. Crop diversification is considered as shifting from comparatively less to more remunerative crops for ensuring sufficient food security (Basavaraja*et al.*, 2016). States have diversified agro-climatic zones and crop diversity; wherein discrepancies do exists on account of crop area and production among different crops. Based on the suitability, crops have different share in area that is spatio-temporal. A cropping pattern is a dynamic process and changes spatio-temporally to meet the cropping area requirements. The cultivable land (15.73 mha) comprises net sown area and current fallow is 51.02% of the total geographical area in Madhya Pradesh (30.83 mha). Shifting of cultivable land to uncultivable land and the low land-man ratio has implications in attaining sustainable crop production (Pavithra*et al.*, 2018). Mtembejiet.al. (2021), analyzed rice production dynamics and found rice as third most stable crop in Tanzania. The judicial utilization of limited natural agricultural resources (like land) is a crucial issue for policymakers to mitigate the challenges of food security for growing populations and sustainable development. Soybean [*Glycine max* (L.) Merrill] is a premier oilseed crop of the rainfed ecosystem of central and peninsular India (Patel *et al.*, 2019b) and cultivated on Vertisols and associated soils. Soybean cultivation in India started in the early1960s; however it was commercially grown since 1970s. With a rapid pace, it covered nearly 11% and 62% of the total *Kharif* area, and among the oilseed crops, respectively in 2020 (Patel and Sharma, 2020). The suitability with various cropping sequences, agro-ecosystems, comparative profitability, and lower cost of cultivation as compared to other competitive crops (Patel *et al.*, 2019a) has led to its rapid expansion from 15 ^{o}N to 25 ^{o}N longitude, covering Madhya Pradesh, Maharashtra, Rajasthan, Chhattisgarh, Telangana, Karnataka and other minor states (Patel *et al.*, 2019b). Soybean area in the country has increased 381 times over the last five decades that is from 0.032 mha in 1970-71 to 12.193 mha in 2019-20.

The study thus was carried out to diagnose the decadal dynamics of land use and cropping pattern to assess the gravity and extent of changes and rate of soybean area expansion in Madhya Pradesh.

MATERIALS AND METHODS

Study area and data source

The time series secondary data on land use pattern and area of major food crops of Madhya Pradesh for the last 50 years period since its commercial cultivation (from 1970 to 2019) was obtained from Directorate of Economics and Statistics, Govt. of India, New Delhi. The study comprises current fallow land, soybean, cotton, sorghum, maize, pearl millet crops, and others (groundnut, finger millets, other oilseeds, pigeon pea and other pulses etc.) and the time duration is divided into five decadal periods.

Analytical tools

a) Percentage Change:

The land-use change detection of different classes has been evaluated by decadal percentage change area, using the function as follows (Sahu*et al.*, 2020)

$$\% \text{ Change} = \frac{Y_t - Y_1}{Y_1} \times 100 \quad \ldots (1)$$

$Y_t$is Value at time'*t*' and Y_1= Initial value

b) Compound growth rate

The rate of area expansion has been worked out by compound growth rates to evaluate growth in area of soybean and related classes under study by the exponential function as follows:

$$Y = ab_e^t \quad \ldots (2a)$$

where,

Yis Dependent variable; ***a***is Intercept; ***b***is Slope and ***t*** is Time period in year

Compound Growth Rate (CGR) in logarithmic form of the equation as

$$logY = \log a + t \log b \quad \ldots (2b)$$

The percentage of CGR has been estimated using

$$CGR(\%) = (Antilogb - 1) * 100 \quad \ldots (2c)$$

c) Markov Chain Technique

"Markov chain is a process that forms the chain of stages with a finite number of states within each stage, if any state at a particular stage is directly dependent on any state of the preceding stage then it is called first-order Markov chain" (Patel *et al.*, 2013). Markov chain assumptions are less stringent and gives more information as compared to regression approach and doesnot need no *apriori* specification of distribution for prediction. Thus, it is a non-parametric approach (Matis*et al.*, 1985). The first-order Markov chain model has been used to assess the share of the land use classes, changing cropping patterns and their future projections (Singh, 2012; Afrin *et al.*, 2020); prediction of crop yields (Patel *et al.*, 2013) and identifying the stable export markets like Groundnut (Jhade*et al.*, 2021).

Markov chain model is based on the assumption in classification of any system or individual into various groups or states and their movement or progress between these states over time and can be considered as a stochastic process (Singh, 2012) or if a set of trials or experiments are analyzed probabilistically (Rao*et al.*, 2005). Markov chain analysis is the application of dynamic programming and is used to detect the structural changes in any system like shifting the crop or land use shares in agriculture where changes occur due to various small forces.

The present study of the movement pattern of cropping area between fallow lands, soybean, and other major crops has been examined using first-order Markov chain and is based on the transition probability matrix (TPM). TPM works on the assumption of constancy *i.e.* the factors affecting the agricultural change in the past will continue to affect it in the future (Gaffney, 1992). TPM is a stochastic matrix with probability elements, Pij, of area transition from ith crop or class in time (or period) t to jth class in time (or period) $t+1$. The TPM Pij, arranged as a square matrix (n x n), where n is the total number of states, is characterized as

a) $0 \leq P_{ij} \leq 1$,

b) $\sum_{i=1}^{n} P_{ij} = 1$, for all (∀) i

The diagonal probability elements P_{ij} (i=j) signify the probability of a crop or land use class retaining its area share over time. However, the off-diagonal probability elements P_{ij}(i≠j), imply the probability of transition from i^{th} class to the j^{th} class. The TPM expresses the overall structure of the transitions or dynamics in any system. The row sum of matrix P_{ij} is 1. The present research has created seven classes viz., current fallow, soybean, cotton, sorghum, maize, pearl millet, and others. It is assumed that the average area of a category in any period is random and depends only on its previous period area (Pavithra*et al.*, 2018). It can be represented algebraically as,

$$E_{jt} = \sum_{i=1}^{n} \left(E_{jt-1} * P_{ij}\right) + e_{jt}$$

Where,

E_{jt}= Area ofj^{th}class during period t

E_{it-1} = Area ofi^{th}class during period t-1

P_{ij}= Transition Probability of area from i^{th} class to j^{th}class.

e_{jt} = Error which is statistically independent of e_{it-1}, and

n= Number of classes (crops or land use class).

The share of the area under crop or land-use class during the time 't' is estimated by multiplying the area of the seven selected classes during the previous time (t-1) with the estimated transitional probability matrix (TPM). The method called minimization of mean absolute deviation (MAD) was used to estimate the TPM by conventional linear programming (LP) framework, as the non-negativity and row sum constraints were satisfied by LP. The LP formulation can be represented as follows (Mahadevaiah*et al.*, 2005).

Min OP* + Ie (sum of the absolute errors)

Subject to,

XP* + V = Y (matrix form of the equation)

GP* = 1 (row sum condition)

P* ≥ 0 (non-negativity condition)

Where, O is a null vector, P* is a column vector of probabilities P_{ij}, I is anappropriately dimensional vector of areas, e is the vector of absolute errors |U|, Y is the vector of areas to each class; X is a block diagonal matrix of lagged values of Y; V is the vector of errors; G is a grouping matrix to add the row elements of P arranged in P* to unity.

RESULTS AND DISCUSSIONS

Decadal Percentage Change

The decadal land-use change detection of soybean and related major competing crops of Madhya Pradesh have been worked out as a percentage change since 1970 (Table 1). The soybean increased by nearly 53 times (from 7.7 to 414.3 thousand hectares) in the first decade (1970-79); about 313, 107, 19 and 11 percent increase in 1980-89, 1990-99, 2000-09, and 2010-19, respectively. However, the trend of percent change has been decreasing. Sorghum area has been continuously decreasing since 1970, similarly to, 'other' category. Cotton was declining from 1970 to 1999 but observed an increase of about 23% in the decade 2000-09 with no change in last decade. The maize area is increasing, but hasdeclined in the second last decade. The percentage of the pearl millet area has been positive (increasing) for two decades. Current fallow land has increased in first two decades. However, later it shows decreasing trend.

The overall land use percentage change since last 50 years as far as soybean is concernedhas been phenomenal i.e. 80342% (from 7.7 – 6194.0 thousand ha); Maize and pearl millet haveincreased by about 134% and 33%, respectively. However, sorghum, cotton andother minor crops, as well as, the current fallow area have decreased significantly by about 95%, 6%, 53%, and 48%, respectively as compared to the first decade (1970) due to the adaptability, high profitability and emergence of high yielding soybean varieties.

Table 1: Decadal Percentage Change of different crops and Current Fallow land in Madhya Pradesh

Year	1970-79	1980-89	1990-99	2000-09	2010-19	Overall% Change
Soybean	5280.52	312.86	106.54	19.19	11.4	**80341.56**
Maize	27.12	12.84	3.22	-10.85	66.51	**134.17**
Pearl Millet	-21.66	-14.58	-20.4	0.12	82.31	**32.98**
Cotton	-11.21	-2.99	-19.79	22.72	0	**-6**
Current Fallow	66.22	6.52	-6.17	-33.1	-25.65	**-47.67**
Sorghum	-1.69	-25.44	-59.38	-31.36	-74.91	**-94.99**
Other	-2.42	-2.56	-6.03	-41.66	-8.08	**-53.14**

Compound Growth Rates

The compound annual growth rates (CAGR) of soybean, related crops, and fallow land in MP were assessed for the last five decades. Soybean growth in Decade-I (1970-79) was remarkably high with 56.83% CAGR; however, the CAGR rate has decreased and turned negative in the last decade (Decade-V). The cotton growth rate is continuously negative except in the fourth decade. The growth rate of sorghum has been decreasing consistently with a pace of nearly -14% CAGR in Decade-V (2010-19). Maize area has increased with a positive growth of about 7% CAGR in the last decade (2010-19), besides a drop in growth in the third and fourth decade (Decade III & IV). This may be due to emergence of high yielding varieties and replacement of traditional crops in new areas. Pearl millet has a negative growth rate in the initial three decades, finally has increased in the last two decades, and has around 8.5% CAGR in the recent decade due to more remunerative prices (increase in minimum support price) for the produce and more nutritional consciousness. Current fallow land has a high growth rate (nearly 4% CAGR) in the first decade but decreased in the next three decades and finally with a minor increase of 1% CAGR during the last decade.This could be due to the fallow lands beingreplaced by major crops and the profitable returns to the farmers. Other crop category has continuous negative growth. The overall growth rates revealed that among the categories, soybean had a rapid growth compared to the rest categories and increased by around 12 percent CAGR. However, sorghum lost its large area share and declined faster than other classes with a negative CAGR of -5.52percent. Soybean might havebeen more remunerative compared to sorghum or other crops like maize and cotton. Except for soybean, maize, and pearl millet; all the rest classes have overall negative growth rates.

Table 2: Decadal Growth Rates of different crops and Current Fallow land in Madhya Pradesh during 1970-2019

Year	1970-79 (Decade-I)	1980-89 (Decade-II)	1990-99 (Decade-III)	2000-09 (Decade-IV)	2010-19 (Decade-V)	Overall CAGR
Soybean	56.83	19.94	8.23	2.32	-0.34	12.06
Cotton	-0.79	-1.02	-1.25	2.39	-0.32	-0.1
Sorghum	-0.91	-2.54	-9.22	-4.45	-13.94	-5.52
Maize	2.51	1.28	-0.23	-0.74	6.59	1.06
Pearl Millet	-2.83	-0.78	-4.32	0.37	8.47	0.39
Current Fallow	4.35	0.93	-1.94	-2.79	1.03	-1.38
Other	0.03	-0.39	-0.71	-3.51	-0.35	-1.91

Markov Chain Analysis

Soybean in Madhya Pradesh has expanded by mainly replacing current fallow land, maize, cotton, and other minor crops. The decadal shifting pattern of the area of these classes has been analyzed using the Markov chain analysis over the last 50 years (from 1970 to 2019). The TPM obtained by Markov chain analysis describes the probability of movement of the cropping pattern of the studied classes from one state to the other. The row elements of TPM give the information about the loss of area share from the particular class to other competing classes. However, column elements provide the extent of gain in area share from competing classes. The diagonal elements signify the retention area share of the previous year's area of the respective class (Jhade*et al.*, 2021). On perusal of the Table 3, it is evident that in 1st decade, 'Soybean' class was the most stable class followed by 'Other' class as they retained 100% and 80% share of the area from the previous year, respectively; cotton, maize, and pearl millet were less stable and retained 44%, 47% and 36% share of the area from the previous year, respectively; and current fallow and sorghum were unstable classes as they did not retain any area share from the previous year. Soybean preserved 100 % area from the previous year and gained 2% area from current fallow class only

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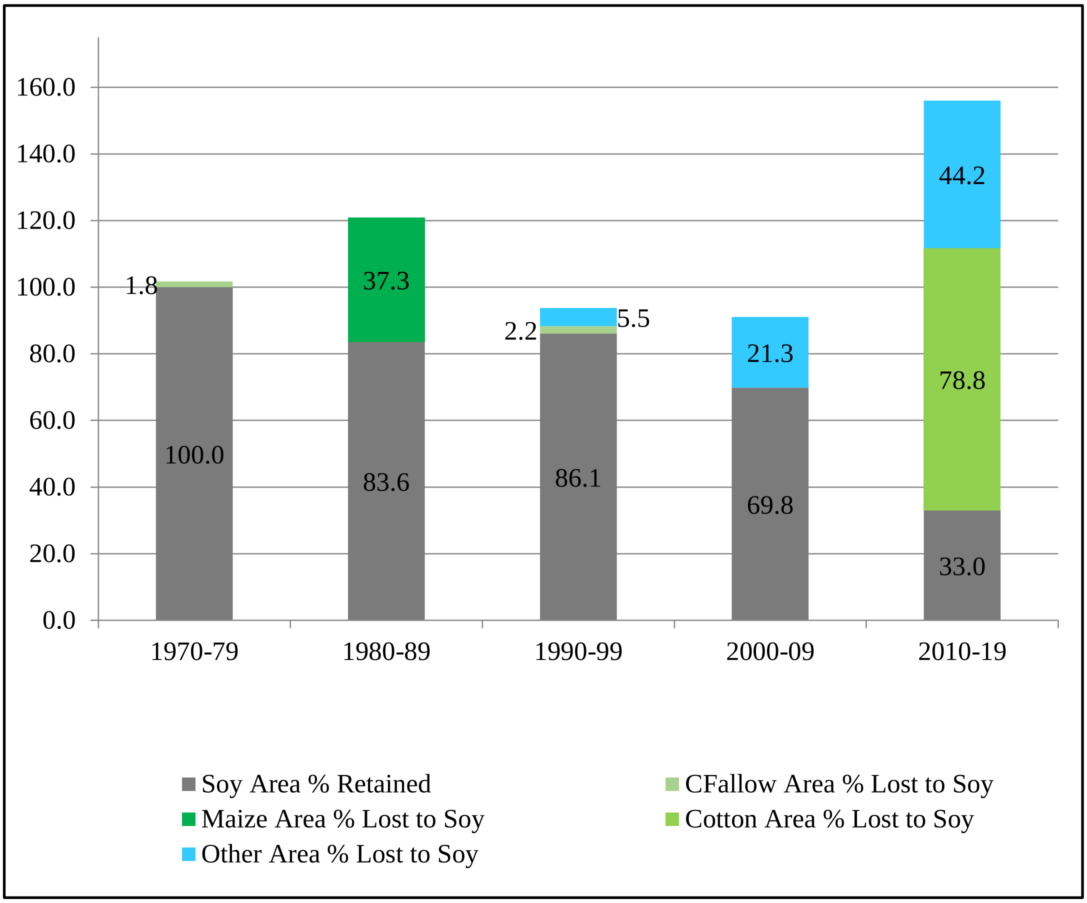

Figure 1: Decadal Soybean percentage of area retained and gained from others

Soybean area increased year by year by replacing the current fallow lands initially (mainly before 1070s), followed by maize, other minor crops, and cotton. The retention of its area share of the previous year gradually declined and became less stable. Although sorghum has lost its 95% area since the beginning but could not be replaced by soybean directly, instead, sorghum first lost its area to other crops, and then those crops area were replaced by Soybean. Sorghum has generally been cultivated by marginal farmers and also in the drought-prone or low water holding areas, hence farmers haven't taken the risk to replace sorghum with soybean directly as sorghum is more tolerant to drought than soybean. Moreover, after the adoption of soybean by large farmers first; marginal and small farmers adapted soybean thereafter.

Table 3. TPM of different crops and current fallow area in Madhya Pradesh during 1970-79 (Decade I)

TPM:1970-79	Current Fallow	Soybean	Cotton	Sorghum	Maize	Pearl Millet
Current Fallow	**0**	0.02	0	0	0.06	0
Soybean	0	**1**	0	0	0	0
Cotton	0	0	**0.44**	0	0	0.17
Sorghum	0	0	0	**0**	0	0
Maize	0.53	0	0	0	**0.47**	0
Pearl Millet	0	0	0	0.64	0	**0.36**
Other crops	0.03	0	0.03	0.12	0.02	0

TPM of decade II (1980-89) revealed that the 'Other crop' class and soybean are the most stable classes retaining 87% and 84% area from previous year share, respectively. Maize and cotton have low retention of 17% and 6% share, respectively; however, current fallow, sorghum, and pearl millet have retained no area and hence are unstable crops. Soybean retained 84% area; meanwhile, it lost its 10% area to current fallow and 6% area to maize; however, gained 37% area from maize (Table 4).

Table 4: TPM of different crops and current fallow area in Madhya Pradesh during 1980-89 (Decade II)

TPM:1980-89	Current Fallow	Soybean	Cotton	Sorghum	Maize	Peal Millet	Other crops
Current Fallow	**0.00**	0.00	0.00	0.67	0.00	0.06	0.27
Soybean	0.10	**0.84**	0.00	0.00	0.06	0.00	0.00
Cotton	0.53	0.00	**0.06**	0.41	0.00	0.00	0.00
Sorghum	0.05	0.00	0.15	**0.00**	0.00	0.00	0.80
Maize	0.23	0.37	0.23	0.00	**0.17**	0.00	0.00
Pearl Millet	0.86	0.00	0.14	0.00	0.00	**0.00**	0.00
Other crops	0.00	0.00	0.00	0.08	0.04	0.01	**0.87**

On scrutinizing Table 5 (TPM of Decade III: 1990-99), it has been observed that 'Other' crops, soybean, and sorghum were the most stable classes with 87%, 86%, and 70% area preserved from preceding years' share; cotton and maize were less stable with 26% and 4% area share retained; however, current fallow and pearl millet were the most unstable crops. Soybean retained 86% area; meanwhile, it lost 4% and 10% area to maize and other crops, respectively; however, gained 2% and 6% area from current fallow and other crops, respectively.

Table 5: TPM of different crops and current fallow area in Madhya Pradesh during 1990-99 (Decade III)

TPM:1990-99	Current Fallow	Soybean	Cotton	Sorghum	Maize	Pearl Millet	Other crops
Current Fallow	**0.00**	0.02	0.00	0.30	0.35	0.02	0.30
Soybean	0.00	**0.86**	0.00	0.00	0.04	0.00	0.10
Cotton	0.74	0.00	**0.26**	0.00	0.00	0.00	0.00
Sorghum	0.20	0.00	0.01	**0.70**	0.08	0.01	0.00
Maize	0.00	0.00	0.00	0.00	**0.04**	0.00	0.96
Pearl Millet	0.11	0.00	0.00	0.00	0.00	**0.00**	0.89
Other crops	0.01	0.06	0.03	0.00	0.03	0.01	**0.87**

On the perusal of TPM of decade IV from 2000-09 (Table 6), it is clear that soybean, other crops, and cotton were the most stable classes with 70%, 58%, and 57% area retained from preceding years' share but less stable than previous periods (1970-79, 1980-89 and 1990-99); in this duration sorghum, pearl millet and current fallows had low stability with 14%, 3% and 1% area share retained from yesteryear; whereas, maize was unstable and retained no area from yesteryear. Although, soybean retained its 70% area but lost its 2% and 28% area to maize and other crops, respectively; however, it replaced or gained 21% area of other crops.

Table 6: TPM of different crops and current fallow area in Madhya Pradesh during 2000-09 (Decade IV)

TPM:2000-09	Current Fallow	Soybean	Cotton	Sorghum	Maize	Pearl Millet	Other crops
Current Fallow	**0.01**	0.00	0.00	0.00	0.09	0.00	0.90
Soybean	0.00	**0.70**	0.00	0.00	0.02	0.00	0.28
Cotton	0.27	0.00	**0.57**	0.00	0.02	0.14	0.00
Sorghum	0.00	0.00	0.00	**0.14**	0.09	0.00	0.77
Maize	0.00	0.00	0.00	0.00	**0.00**	0.00	1.00
Pearl Millet	0.00	0.00	0.00	0.00	0.97	**0.03**	0.00
Other crops	0.05	0.21	0.03	0.06	0.06	0.01	**0.58**

The last decade (2010-19) TPM (Table 7) showed that sorghum has high stability of 74% area retained from the preceding year. However, other crops (49%), maize (42%), soybean (33%), current fallows (21%), and cotton (21%) have low stability. During this period, pearl millet lost its complete area and was found to be most unstable. Although soybean retained only 33% of its area share of the previous year and lost its 1%, 8%, and 58% area share to cotton, maize, and other crops, respectively; but, it captured 79% and 44% area of cotton and other crops, respectively. In the last decade, soybean gained a large areapercentage (123%) of cotton andother minor crops.

Table 7: TPM of different crops and current fallow area in Madhya Pradesh during 2010-19 (Decade V)

TPM:2010-19	Current Fallow	Soybean	Cotton	Sorghum	Maize	Pearl Millet	Other crops
Current Fallow	**0.21**	0.00	0.24	0.10	0.00	0.00	0.45
Soybean	0.00	**0.33**	0.01	0.00	0.08	0.00	0.58
Cotton	0.00	0.79	**0.21**	0.00	0.00	0.00	0.00
Sorghum	0.00	0.00	0.00	**0.74**	0.00	0.00	0.26
Maize	0.12	0.00	0.00	0.00	**0.42**	0.24	0.22
Pearl Millet	0.00	0.00	0.00	0.00	1.00	**0.00**	0.00
Other crops	0.03	0.44	0.04	0.00	0.00	0.00	**0.49**

Over last five decades, it has been observed that sorghum and 'other crops' area has been declining in Madhya Pradesh and the more remunerative crops have replaced them with new higher yielding varieties. Low yield of sorghum and changing agro-climatic conditions such as soil, temperature and rainfall distribution, i.e., the physical conditions of the region had led to decline to sorghum area (Rao *et al.*, 2005). Sorghum was unstable and 'other crops' were highly stable initially, however, in recent decade sorghum slowly becoming stable and 'other crops' showed less stability. Cotton, maize, and pearl millet are less stable during the period of fifty years. The continuous decline of sorghum during the last five decades is mainly due to reduced consumption and less remuneration. The continuous reduction in the demand of sorghum growing to the reduced consumption has led to the growers to move to more remunerative crops like groundnut, pigeon pea, and other oilseeds.

CONCLUSION

The cropping pattern change during a particular period signifies the agricultural development of the area. On perusal of growth rate and cropping pattern change analysis of major *rainfed* crops, it is revealed that akin to other high valued crops, soybean area has also increased significantly and was highly stable initially, but slowly becoming less stable due to change in climatic pattern. Cotton, sorghum, maize, pearl millet, and other minor crops are the major competing crops of soybean in Madhya Pradesh. Sorghum was generally been cultivated by marginal farmers and also in the drought-prone areas as it is more drought tolerant. It was found that sorghum and 'Other Crops' area has been rapidly declining since the last fifty years in Madhya Pradesh. Initially, soybean started growing on the current fallow land but later it replaced other major competing crops besides fallow lands. Hence, in the context of the above it can be concluded that soybean area may increase nearly 20-25% (12% per decade) during next two decades due to several reasons i.e. having more stability than other crops; area gain from other competing crops; more remunerative; increasing adaptability and increased consumption in the form of food as well as, due to development of climate resilient varieties. Hence, to achieve self-sufficiency in oilseeds in the country, suitable promotional policies have to be envisioned; efforts for area expansion and off-season production need to be made.

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AUTHOR CONTRIBUTIONS

Author Contributions: Conceptualization, R.M.P. and N.K.; methodology, R.M.P. soft-ware, R.M.P. and K.B.; validation, R.M.P. and P.S.; formal analysis, R.M.P.; investigation, A.R. and N.K.; resources, N.K.; data curation, R.M.P.; writing—original draft preparation, R.M.P.; writing—review and editing, R.M.P., P.S. and A.R.; visualization, R.M.P. and N.K.; supervision, N.K.; project administration, R.M.P.; funding acquisition, N.K. All authors have read and agreed to the published version of the manuscript.

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LIABILITIES	AMOUNT	ASSETS	AMOUNT
Capital Fund		**Fixed Assets**	
Opening Balance	5,521,559	Computer & Printer	9
Add : Life Membership	25,000	Less :- Depreciation	4
Add : Surplus of the year	356,896		5
	5,903,455		
		Furniture & Fixture	7,953
		Less :- Depreciation	795
			7,157
		Investments	
		Fixed Deposit	1,098,800
		Accrued Interest	56,829
		Mutual Fund (in Canara Bank)	4,333,860
		Current Assets	
		TDS Recievable	23,427
		Bank Balance - Canara Bank	383,376
TOTAL ₹	**5,903,455**	**TOTAL ₹**	**5,903,455**

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED ON 31.03.2023

EXPENDITURE	AMOUNT	INCOME	AMOUNT
Soya Mahakumbh Expenses	2,125,366	Soya Mahakumbh Sponsorship	2,208,112
AGM Expenses	711,077	AGM Registration Fees	960,000
Publication Expenses	54,939	AGM Sponsorship	50,000
Web Designing Expenes	9,000	Membership Fees	3,900
Postage & Stationery	20,660	Interest Received	
		- On Fixed Deposit	63,144
Legal & Professional Fees	8,839	- On Saving Account	20,298
Bank Charges	853		
Income Tax (TDS of previous years)	17,025		
Depreciation	799		
Excess of Income over Expenditure	356,896		
TOTAL ₹	**3,305,454**	**TOTAL ₹**	**3,305,454**

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