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Editorial

Biological Sciences cover a wide range of themes ranging from core molecular events occurring in a living cell to engineering disease resistant crops. The fact that the cellular events are extremely complex in both prokaryotes and eukaryotes, gaining insights on the molecular mechanisms is need of the hour. For instance, DNA which is considered as the biomolecules comprising necessary information for sustenance of life forms can be manipulated so as to modify the entire organism with extrapolated characteristics, which would be beneficial for the society. Understanding the role of nucleic acids and their protein counterparts' synthesized post central dogma events is essential so as to identify the potential role of any gene. Even though many sophisticated biophysical characterization techniques are available, dissecting the importance of a given biomolecule is difficult. For example, gene knockout, gene knockdown, gene silencing are considered as the best approaches to study the function of gene(s). However, the tricky part is minimizing the off-targets, so that the function of the gene of interest can be suitably studied. Likewise, next generation sequencing and genome editing approaches play a key role in unraveling the molecular mechanisms in eukaryotes. Multiple variants of SARS-CoV2 were identified by advanced next generation sequencing, which ultimately led researchers to develop drugs to restrict the viral replication. Besides the eukaryotes, prokaryotes also play a significant role in maintaining the ecological sustainability primarily by getting involved in multiple phenomena including biodegradation, microbes-assisted pollutant remediation and many more. Currently, agricultural development is the primary agenda of our Nation. Nitrogen-fixation via microbe, supplements adequate nutrition to plants resulting in better productivity. Plants are sessile, therefore constantly exposed to pathogens resulting in huge loss. It is estimated that the plant productivity suffers a loss of 40% annually across globe highlighting the urgency of developing strategies to combat the pathogens. Understanding the pathogens and effectors released

by them to injure plants is the key in identifying the gene targets, that can potentially facilitate in designing pathogen-resistant crops.

This issue includes key biological concepts such as fundamental research in ecology, agricultural sustainability, genetics, ecology and physiology from cellular to molecular level, messenger RNA regulation, gene editing technologies with an outcome-based approach, aligning with the achievement of various SDGs as framed by the United Nations.

Chief Editor

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Articles

Effect of irrigation and dates of sowing on growth, yield attributes and yield of sunflower (Helianthus annuus L.) in coastal saline soil of Eastern India

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Tanuj Kumar Mandal[1]*, Pijush Das[1] and Mousumi Mondal[1]

Abstract

The field experiment was carried out at the University Farm, Sarisha, The Neotia University, South 24 Parganas, West Bengal during two consecutive winter seasons of 2020-21 and 2021- 22 to study the effect of irrigation and dates of sowing on growth, yield attributes and yield of sunflower in coastal saline soil of Eastern India. The soil of the experimental field was fine in texture and clayey in nature. The experiment was laid out in a split-plot design, replicated thrice, having 3 irrigation treatments in the main plots viz., I_1 – irrigation at bud stage, I_2 - irrigation at bud stage and grain formation stage, I_3 - irrigation at bud

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stage, flowering stage and grain formation stage and 4 dates of sowing in sub-plots viz. D_1 – 3rd week of November, D_2 – 4th week of November, D_3 – 1st week of December and D_4 – 2nd week of December, respectively. The crop variety was *LG 50801*. The respective fertilizer dosages of sunflower of N: P_2O_5: K_2O @ 80: 60: 40 kg ha^{-1} was considered as the recommended dose of the crops. Among all the levels of irrigation, I_3 *i.e.*, irrigation at bud stage, flowering stage and grain formation stage recorded the highest values of all yield attributes and seed yield (27.46 q ha^{-1}) of sunflower followed by irrigation at bud stage and grain formation stage *i.e.* I_2 and the lowest values were recorded under irrigation at bud stage *i.e.*, I_1 treatment. Maximum oil content (43.12 %) and oil yield (11.84 q ha^{-1}) were recorded under I_3 *i.e.*, irrigation at bud stage, flowering stage and grain formation stage. Among the dates of sowing D_1 *i.e.*, sowing in the third week of November recorded the highest values of all the yield attributes, seed yield, oil content and oil yield of sunflower. Delayed sowing drastically reduced the seed yield, oil content and oil yield of sunflower.

Introduction

Sunflower (*Helianthus annuus* L.) is considered one of the important essential oilseed crops in India. It is commonly known as "Surajmukhi" in the country. It is the third most important edible oil seed crop in the world preceded by soybean and rapeseed (Sandeep Kumar *et al*., 2019; Sial *et al*., 2022). The total area under sunflower cultivation is estimated 26.2 M ha all over the globe with a net production of 47.3 M tonnes in 2016 (FAO, 2017). But, in India, it is grown in only 0.29 m ha area with very low production of 0.21 m tones (DAC & FW, 2018). It indicates that there is ample scope to increase the area under sunflower cultivation. There is an enormously growing popularity of sunflower oil as cooking oil or vegetable oil because of its so many health benefits. Sunflower oil has high oxidative stability and includes significant levels of vitamin E in the form of alpha-tocopherol. It also contains a lot of

linoleic acid (64%) which helps to prevent cholesterol build-up in the heart and coronary arteries.

It is mainly cultivated in the temperate region where it can adapt easily and perform well to a variety of climatic and soil conditions (Canavar *et al*., 2010; Demir, 2016; Demir and Basalma, 2018). It is a photo insensitive crop and can be grown successively throughout the year in different parts of the country under rainfed conditions. However, it can also respond favourably to applied irrigation water in terms of crop growth and yield in situations where rainfall and soil water supplies are limited, particularly during the dry months of the year.

Sunflower, even though tolerant to mild water stress, is capable of producing higher yields when an adequate amount of irrigation water is applied (Karam *et al*., 2007). However, the quantity and timing of irrigation are two crucial factors that regulate the effective utilization of applied water and optimize the yield of crops (Sezen *et al*., 2011). Water shortages have a considerable negative impact on capitulum diameter, yield plant^{-1} and seed test weight during any one of the reproductive sub-stages such as blooming, anthesis and seed filling (Reddy *et al*., 2004 and Geetha *et al*., 2012). Therefore, the production goals must change from achieving potential yield per unit of land to potential yield per unit of water due to the limited availability of fresh water for raising agricultural crops. To increase productivity per available drop of water, efforts must now be made to harness the water that is now accessible and use it effectively (Solaimalai *et al*., 2005). Improvement of irrigation practices must, therefore, receive more attention if crop production is to be increased and productivity levels are to be maintained for a longer period of time (Kalpana and Anita, 2014).

On the other hand, under varying climatic conditions, the sowing date has a significant influence on sunflower seed production and oil content.

Delay in sowing reduces sunflower seed yield because plants produce fewer, lighter seeds (Siddique et al., 2002). Again, in the case of delayed sowing soil water availability is typically limited during the flowering and seed development stage, which are critical times for seed filling. Lack of water during these stages reduces the soil's ability to supply nutrients for reproductive growth, which in turn lowers seed production (Ali *et al*., 2012). Additionally, several studies also revealed that delayed sowing in general decreases crops yield during dry months, particularly under rainfed conditions (Nihal, 2010; Ahmed *et al*., 2015).

Therefore, in order to meet the demand for vegetable oil and enhance the production of sunflower in dry conditions, it is necessary to determine the effects of irrigation schedule and sowing date on yield parameters and grain yield of hybrid sunflower cultivars during dry season of the year. Improved management strategies can be established to provide better results when sunflower is not fully irrigated by having a better grasp of how sunflower growth and development are affected by water availability at several readily recognizable growth stages and varying planting dates. The purpose of this study is to determine the effects of irrigation water application on the seed yield, oil content, and other yield components of hybrid sunflower cultivars with four different planting dates.

Materials and Methods

The field experiment was carried out at the University Farm, Sarisha, The Neotia University, South 24 Parganas, West Bengal during two consecutive winter seasons of 2020-21 and 2021- 22. The farm is located at the south of the tropic of cancer, 22° 262 N latitude and 88° 192 E longitudes. The soil of the experimental field was fine in texture and clayey in nature. The experiment was laid out in a split-plot design, replicated thrice, having 3 irrigation treatments in the main plots viz. I_1 – irrigation at bud stage, I_2 - irrigation at bud stage and grain

formation stage, I_3 - irrigation at bud stage, flowering stage and grain formation stage and 4 dates of sowing in sub-plots viz. D_1 – 3rd week of November, D_2 – 4th week of November, D_3 – 1st week of December and D_4 – 2nd week of December, respectively. The crop variety was *LG 50801*. The respective fertilizer dosages of sunflower of N: P_2O_5: K_2O @ 80: 60: 40 kg ha^{-1} was considered as the recommended dose for the crop. A full dose of P_2O_5 and K_2O @ 30: 60: 40 kg ha^{-1} were applied as basal. Rest amount of N (50 kg ha^{-1}) was equally splitted into two, of which 1st was applied 30 days after sowing (DAS) and 2nd was applied at 50 DAS. The crop was harvested from 3 m × 2m net area of each plot (gross plot size 6 m × 3 m), discarding 1 m around to avoid the border effect and threshed. Total nitrogen content of soil was determined in percentage, according to 7 modified *Kjeldahl* methods as described by Jackson (1973). Available P_2O_5 was determined by Bray and Kurtz (1945) method, as described by Jackson (1973) and available K_2O was determined by using flame photometer (Muhr *et al.* 1965). The data were subjected to statistical analysis using analysis of variance method (Gomez and Gomez, 1984) and the significance of different sources of variations were tested by error mean square using Fisher and Snedecor's 'F" test at a probability level of 0.05.

Results and Discussion

Levels of irrigation significantly influenced the number of head diameter (cm.), head weight (g), number of seed $head^{-1}$, 100 seed weight (g) and seed yield (q ha^{-1}) of sunflower. Among all the levels of irrigation I_3 *i.e.*, irrigation at bud stage, flowering stage and grain formation stage recorded the highest values of all yield attributes and seed yield (27.46 q ha^{-1}) of sunflower followed by irrigation at bud stage and grain formation stage *i.e.*, I_2 and the lowest values were recorded under irrigation at bud stage *i.e.*, I_1 treatment (Table 1). Proper moisture in the root zone has helped in the translocation of nutrients as well as maintenance of proper hydration for the proper functioning of biochemical reactions resulting

in maximum values under irrigation at the bud stage, flowering stage and grain formation stage *i.e.* I_3. Maximum oil content (43.12 %) and oil yield (11.84 q ha^{-1}) were recorded under irrigation at bud stage, flowering stage and grain formation stage.

Among the dates of sowing D_1 *i.e.*, sowing at the thrid week of November recorded the highest values of all the yield attributes, seed yield, oil content and oil yield of sunflower. Delayed sowing drastically reduced the seed yield, oil content and oil yield of sunflower. Sunflower productivity is mainly determined by the prevailing weather conditions throughout its life cycle (Kaleem *et al*., 2011) and the decreased seed yield due to delayed sowing might be due to a decrease in yield components (Siddique *et al*., 2002).

Table 1: Effect of irrigation and dates of sowing on yield attributes and yield of sunflower (Pooled data)

Treatments	Head diameter (cm.)	Head weight (g)	Number of seeds $head^{-1}$	Seed length (mm)	Seed diameter (mm)	100 seeds weight	Seed yield (q ha^{-1})	Oil content (%)	Oil yield (q ha^{-1})
Irrigation levels									
I_1	11.35	66.27	677.85	9.50	5.34	48.73	25.74	42.37	10.90
I_2	12.35	68.78	722.50	9.71	5.54	49.93	26.42	42.72	11.28
I_3	13.44	72.25	791.77	9.96	5.69	51.52	27.46	43.12	11.84
S. Em.	0.028	0.075	0.923	0.015	0.005	0.052	0.021	0.036	0.004
C.D. (P= 0.05%)	0.080	0.209	2.562	0.043	0.013	0.145	0.059	0.101	0.013
Date of Sowing									
D_1	12.60	69.73	757.93	9.84	5.60	50.94	26.98	42.93	11.58
D_2	12.42	69.16	735.30	9.74	5.55	50.14	26.67	42.79	11.41
D_3	12.33	68.87	718.63	9.69	5.50	49.85	26.31	42.64	11.22
D_4	12.17	68.65	710.96	9.62	5.43	49.31	26.20	42.59	11.15
S. Em. ±	0.036	0.077	1.160	0.018	0.008	0.080	0.024	0.034	0.015
C.D. (P= 0.05%)	0.077	0.162	2.438	0.038	0.016	0.168	0.051	0.072	0.032

Conclusion

The study revealed that winter sunflower can be grown in coastal areas of Eastern India with three irrigations at bud formation, flowering and grain formation stages and the crop should be sown on the third week of November to obtain higher growth and productivity as well as edible oil yield. Delayed sowing significantly reduced the growth attributes, yield attributes, yield and oil content of sunflower.

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Comparative study on performance of different organic nutrient management practices under pigeonpea (Cajanus cajan (L.) Millsp.) production system

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Abstract

To enhance the productivity of pigeonpea, combined application of composts and biofertilizers along with nitrogen fertilizer is an appealing alternative under reduced availability of FYM. Accordingly, a field experiment was designed at Main Agricultural Research Station, Dharwad during *Kharif* 2016 under medium deep black soils. Randomized block Design with nine treatments (T_1 - T_6: sesame, wheat, pigeonpea, cotton and maize residue composts and FYM along with biofertilizers + RDF, respectively; T_7: FYM without biofertilizers + RDF, T_8 - RDF + biofertilizers

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and T_9 - RDF alone) and three replications were employed for comparative study of residue based composts and FYM along with biofertilizers and RDF on performance of pigeonpea. Significantly higher seed yield (2158 kg ha^{-1}), haulm yield (5176 kg ha^{-1}) and harvest index (27.12 %) was recorded with FYM + biofertilizers + RDF as compared to RDF + Biofertilizers (1387, 4313 kg ha^{-1} and 24.48 %, respectively) and RDF alone (1283, 4183 kg ha^{-1} and 24.06 %, respectively). Similarly, constant increase of the growth parameters like plant height, number of secondary branches and TDMP was observed from 60 DAS towards harvest of the crop. Besides this, significant increase of SPAD value was observed from 60 DAS upto 90 DAS and then gradually declined towards the harvest of the crop. From this one year of study can be concluded that, application of FYM, sesame, wheat, pigeonpea, cotton and maize residue composts along with biofertilizers and RDF improved seed yield to the tune of 16.8, 14.66, 15.48, 15.11, 15.68 and 13.32 % over RDF alone.

Keywords: Pigeonpea, crop residue compost, Biofertilizers, Compost culture, SPAD, Pigeonpea yield

Introduction

An estimated amount of 679 million tonnes of crop residues were generated in India and these residues were an important source of nutrients for subsequent crops. Around 312.5 million tonnes of residues are generated from the 10 major crops (rice, wheat, sorghum, pearl millet, barley, finger millet, sugarcane, potato tubers and pulses), which have a nutrient potential of about 6.46 million tonnes of NPK. It has been estimated that all animal excreta can potentially supply 17.77 million tonnes of NPK, where the present usage is only 33.3 % (IARI, 2021). The second most important pulse crop of India is pigeonpea, which plays a vital role in daily diet. Pigeonpea is highly nutritious and rich in protein (21.7 %), carbohydrates, fibres and minerals. Among the

major countries growing pigeonpea, India ranks first with about 75 % of the world area and 67 % of production. It occupies an area of about 3.5 million ha with a total production of 4.23 million tonnes, with an average productivity of 753 kg ha^{-1} (DES, GOI., 2022).

The yield of pigeonpea is influenced by several factors like, agronomic, pathogenic, entomological, genetic factors and their interaction with the environment. The main reason for low and unstable yield of pigeonpea is an inadequate and imbalanced nutrient application of nitrogen and phosphorous. Because of increasing the cost of inorganic fertilizers, decreased soil fertility, reduced environmental health concerns due to pesticide usage and due to increased premium prices for organic products farmers are much more interested in cultivating organic farming (Ramesh 2005).

Now-a-days compost culture (mixture of lingo-cellulolytic microbes' *viz.,Trichoderma viridae, Phanerochaete chrysosporium, Pleurotus, Aspergillus sidowia*) is known for its efficiency in converting crop residues into good compost within 75-90 days. To enhance the productivity of pigeonpea, use of balanced fertilization by application of organic manures and chemical fertilizers along with biofertilizers *viz*., *Rhizobium*, PSB and Mycorrhiza have a stimulatory effect on increasing the nutrient availability and improve the yield parameters. With this background, the field experiment was conducted for "Comparative study on the performance of different organic nutrient management practices under pigeonpea (*Cajanus cajan* (L.) Millsp.) production system".

Materials and Methods

Location: The experiment was conducted at the Main Agricultural Research Station, University of Agricultural Sciences Dharwad, which is situated at 15°26' N latitude and 75°01' E longitude and at an altitude of 678 m above mean sea level. Soil type of experimental site was medium black clay soil and low in nitrogen at 0-30 and 30-60 cm depth (250.6

and 217.00 kg ha^{-1}, respectively), medium in phosphorus at 30 cm depth (24.32 kg ha^{-1}) low in phosphorus in 30-60 cm depth (18.32 kg ha^{-1}) and high in potassium at both 0-30 and 30-60 cm depth (398 and 370 kg ha^{-1}, respectively). The rainfall received during 2016 was 531.2 mm which was much less than the normal rainfall.

Experimental treatments and design: The treatments comprised of T_1:Sesame residue compost at 6 t ha^{-1} + RDF + Biofertilizers, T_2:Wheat residue compost at 5.4 t/ha + RDF + Biofertilizers, T_3:Pigeonpea residue compost at 4 t/ha + RDF + Biofertilizers, T_4:Cotton residue compost at 7.1 t/ha + RDF + Biofertilizers, T_5:Maize residue compost at 6.4 t/ha + RDF + Biofertilizers, T_6:FYM at 6 t/ha + RDF + Biofertilizers, T_7:FYM at 6 t/ha + RDF + without Biofertilizers, T_8:RDF + Biofertilizers and T_9:RDF alone. The five crop residues (sesame, wheat, pigeonpea, cotton and maize) were composted in pits for 90 days using compost culture (*viz., Trichoderma viridae, Phanerochaete chrysosporium, Pleurotus, Aspergillus sidowia*). These composts were analysed for NP and K contents and these were applied on N equivalent basis as that of FYM. Recommended dose of fertilizer is 25:50 kg N:P_2O_5 ha^{-1}. The experiment was laid out in Randomized Complete Block Design in with three replications. Nitrogen was applied through DAP as starter dose (25:50) as per the treatments. Composts were applied before 15 days of sowing. Seeds were treated using *Rhizobium* and PSB @ 1,250 g/ha and mycorrhizae was applied to soil @ 20 kg/ha at the time of sowing. Two seeds per hill were dibbled 5 cm deep in furrows at a spacing of 120 cm x 20 cm and the variety used was TS-3(R). The crop was sown during the second week of July. The crop was harvested at its physiological maturity, and grain and straw yields of pigeonpea were recorded at harvest.

Collection of experimental data: Five plants were randomly selected and tagged in the net plot of all the plots. The plant height was measured from the ground level to the tip of the main stem and recorded at 60, 90 and 120 DAS and at harvest. The average height of the randomly

selected five plants was calculated and expressed as plant height in centimeters. The number of branches emerging from the primary branches was counted at 60, 90 and 120 DAS and at harvest. The average of five plants was expressed as a number of secondary branches plant^{-1}. Five plants from the net plot were selected randomly and they were cut close to the ground. The plant parts were separated into leaves, stem and pods at different growth stages. Then they were dried at 70°C in hot air oven until the constant weight was attained and the oven dry weight of different plant parts were recorded separately at 60, 90 and 120 DAS and at harvest and expressed in g plant^{-1}. Using SPAD meter SPAD value was recorded at 60, 90 and 120 DAS and at harvest.

The seed yield obtained from each net plot area was used for calculating seed yield ha^{-1}. Plants from the net plot after threshing were dried and their weight was recorded per plot area. From this stalk yield ha^{-1}was calculated. The harvest index was estimated as per the formula suggested by Donald (1962).

$$HI = \frac{\text{Economic Yield (seed yield)(kg/ha)}}{\text{Biological yield (seed yield + stalk yield)(kg/ha)}} \times 100$$

Statistical analysis of data: The data of the experiment was analyzed statistically following the procedure described by Gomez and Gomez (1984). The level of significance used in 'F' test was p=0.05.

Results and Discussion

Seed yield, stalk yield and harvest index: Significantly higher seed yield (2158 kg ha^{-1}), stalk yield (5176 kg ha^{-1}) and harvest index (27.12 %) was recorded with FYM + biofertilizers + RDF as compared to RDF + Biofertilizers (1387, 4313 kg ha^{-1} and 24.48 %, respectively) and RDF alone (1283, 4183 kg ha^{-1} and 24.06 %, respectively). However, it was

found on par with all other residue-based composts (cotton, wheat, pigeonpea, sesame and maize) + biofertilizers + RDF (Table 1).Whereas, significantly lower seed yield was recorded by RDF alone (1,283 kg ha^{-1}) and RDF with biofertilizers (1,387 kg ha^{-1}) (Table 1). The higher yield was due to the increased yield attributes. These results are in close confirmation with the findings of Reddy *et al*. (2011), who reported that combined application of FYM, biofertilizers and RDF recorded higher seed yield of pigeonpea than RDF alone treatment.The increase in seed yield might be due to the positive effect of combined application of organic fertilizers, inorganic fertilizers and biofertilizers on better root development, which resulted in more nutrient uptake. These micro-organisms also produce vitamins and plant growth-promoting substances for the betterment of plant growth. Organic manures not only release nutrients slowly but also prevent the losses of leaching (Arshad *et al*., 2004 and Anup Das *et al*., 2010). The increase in stalk yield in these treatments could be attributed to better plant growth, as evidenced by the increased number of primary and secondary branches (Table 2). However, significantly lower stalk yield was recorded RDF alone (4,183 kg ha^{-1}) and RDF with biofertilizers (4,313 kg ha^{-1}), which might be due to low nutrient availability to the crop thought its growth period.These results of the current study also confirmed by the findings of Jat and Ahlawat (2010).

There was no significant difference among the treatments with respect to harvest index. Numerically superior harvest index (27.12 %) was recorded in FYM with biofertilizers as compared to RDF alone (24.06 %). However, it was on par with cotton, wheat, pigeonpea, sesame and maize residue composts along with biofertilizers and FYM without biofertilizers (26.48, 25.69, 25.44, 25.33, 25.08, 24.48 and 25.96 %, respectively).

Table 1: Seed yield, stalk yield and harvest index of pigeonpea as influenced by different crop residue-based composts, FYM and biofertilizers

Treatments	Seed yield (kg ha^{-1})	Stalk yield (kg ha^{-1})	Harvest index
T_1: RDF_{NPK} + Sesame residue compost @ 6 t ha^{-1} + Biofertilizers	1882	4713	25.33
T_2: RDF_{NPK} + Wheat residue compost @ 5.4 t ha^{-1} + Biofertilizers	1986	4857	25.69
T_3: RDF_{NPK} + Pigeonpea residue compost @ 4 t ha^{-1} + Biofertilizers	1939	4794	25.44
T_4: RDF_{NPK} + Cotton residue compost @ 7.1 t ha^{-1} + Biofertilizers	2012	4959	26.48
T_5: RDF_{NPK} + Maize residue compost @ 6.4 t ha^{-1} + Biofertilizers	1709	4710	25.08
T_6: RDF_{NPK} + FYM @ 6 t ha^{-1} + Biofertilizers	2158	5176	27.12
T_7: RDF_{NPK} + FYM @ 6 t ha^{-1}	1960	4886	25.96
T_8: RDF_{NPK} + Biofertilizers	1387	4313	24.48
T_9: RDF_{NPK}	1283	4183	24.06
S.Em±	**155**	**191**	**0.99**
$CD_{p\leq 0.05}$	**464**	**573**	**NS**

*Recommended dose of fertilizer is 25:50 kg $N:P_2O_5$ ha^{-1}.

Growth parameters and SPAD value: FYM + biofertilizers + RDF recorded significantly higher plant height (98.67, 139, 163 and 165 cm, respectively) (Table 2), number of secondary branches (8.03, 22.17, 24.00 and 25.26, respectively) (Table 2) and total dry matter production (23.80, 39.47, 154.46 and 186.59 g $plant^{-1}$, respectively) at 60, 90, 120 DAS and at harvest (Table 3) as compared to RDF alone (93.67, 117.67, 139.63 and 146 cm of plant height; 6.87, 15.94, 16.07 and 16.44 number of secondary branches; 19.52, 28.13, 125.30 and 142.78 g $plant^{-1}$ of total dry matter production at 60, 90, 120 DAS and at harvest,

respectively). However, it was found on par with all residue-based composts (cotton, wheat, pigeonpea, sesame and maize) + biofertilizers + RDF (Table 2). Besides this, a significant increase in SPAD value was observed from 60 DAS upto 90 DAS and then gradually declined towards the harvest of the crop. Significantly higher SPAD value was recorded with FYM + biofertilizers + RDF at 60, 90, 120 DAS and at harvest (22.45, 49.02, 41.87 and 36.10, respectively) as compared to RDF alone (19.83, 43.66, 36.81 and 32.26, respectively). Similarly, all residue-based compost treatments were found on par with it (Table 3).

Yield attributes indirectly depend on growth attributes like plant height, number of secondary branches, chlorophyll content, and total dry matter accumulation. The pre-requisite for getting higher yield in any crop depends upon total dry matter production (TDMP) and its accumulation in reproductive parts which in turn influences the yield components. The total dry matter accumulation of pigeonpea differed significantly due to five different residue-based composts of sesame, wheat, pigeonpea, cotton maize along with biofertilizers and FYM with biofertilizers and RDF (Table 3). The superiority of these treatments as compared to RDF alone and RDF with biofertilizers was due to better growth of the plant as evidenced by plant height and higher number of secondary branches plant^{-1}. These findings are in confirmation with Adebayo *et al.* (2013) reported that the nutrient content of compost materials is higher as compared to RDF. The results clearly indicate that all residue-based composts along with biofertilizers and RDF can be used as alternatives to FYM and will be better treatment, than RDF alone and RDF with biofertilizers in promoting higher plant height, a higher number of primary and secondary branches plant^{-1} and total dry matter accumulation plant^{-1}, which in turn favorably influenced the yield components and yield of pigeonpea.

Table 2: Plant height (cm) and no. of secondary branches per plant of pigeonpea as influenced by different crop residue-based composts, FYM and biofertilizers

Treatments	Plant Height (cm)				No. of secondary branches plant^{-1}			
	60 DAS	90 DAS	120 DAS	Harvest	60 DAS	90 DAS	120 DAS	Harvest
T_1	95.89	131.44	154.33	157.67	7.23	19.67	22.00	22.97
T_2	95.78	133.00	159.00	160.44	7.37	20.20	22.81	23.45
T_3	95.67	131.44	156.48	159.11	7.33	19.93	22.33	23. 03
T_4	98.33	135.67	161.00	161.33	7.97	21.13	23.53	24.03
T_5	95.11	130.78	152.00	156.96	7.20	19.51	21.50	22.94
T_6	98.67	139.00	163.33	165.37	8.03	22.17	24.00	25.26
T_7	97.00	134.33	158.00	160.78	7.67	20.33	23.47	23.89
T_8	94.89	126.33	148.00	151.48	6.97	16.17	16.90	17.17
T_9	93.67	117.67	139.63	146.00	6.87	15.94	16.07	16.44
S.Em±	3.24	3.76	4.50	3.13	0.28	0.79	1.10	0.89
CD at 5 %	NS	11.35	13.50	9.40	NS	2.37	3.39	2.67

Note: T_1 : Sesame residue compost at 6 t ha^{-1} + RDF + Biofertilizers, T_2 : Wheat residue compost at 5.4 t ha^{-1} + RDF + Biofertilizers, T_3 : Pigeonpea residue compost at 4 t ha^{-1} + RDF + Biofertilizers, T_4 : Cotton residue compost at 7.1 t ha^{-1} + RDF + Biofertilizers, T_5 : Maize residue compost at 6.4 t ha^{-1} + RDF + Biofertilizers, T_6 : FYM at 6 t ha^{-1} + RDF + Biofertilizers, T_7 : FYM at 6 t ha^{-1} + RDF + without Biofertilizers, T_8 : RDF + Biofertilizers, T_9 : RDF alone

*Recommended dose of fertilizer is 25:50 kg N:P_2O_5 ha^{-1}.

Table 3: Total dry matter production (TDMP) per plant and SPAD value of pigeonpea as influenced by different crop residue-based composts, FYM and biofertilizers

Treatments	TDMP (g plant^{-1})				SPAD value			
	60 DAS	90 DAS	120 DAS	Harvest	60 DAS	90 DAS	120 DAS	Harvest
T_1	22.44	36.58	147.37	177.22	21.27	46.35	38.69	34.10
T_2	22.86	37.49	149.46	179.58	21.80	46.97	41.33	35.00
T_3	22.63	36.96	148.21	177.84	21.80	46.85	41.17	34.67
T_4	23.74	38.69	153.86	182.07	21.95	47.83	41.65	35.95
T_5	22.30	36.44	147.12	176.89	21.18	46.61	38.52	34.07
T_6	23.80	39.47	154.46	186.56	22.45	49.02	41.87	36.10
T_7	23.62	38.30	152.19	181.55	22.23	47.69	41.44	35.68
T_8	21.16	29.96	130.50	151.33	20.10	44.50	37.10	33.14
T_9	19.52	28.13	125.30	142.78	19.83	43.66	36.81	32.26
S.Em±	0.66	1.05	3.01	3.29	0.65	1.02	1.18	0.80
CD at 5 %	1.97	3.15	9.03	9.86	NS	3.07	3.55	2.39

Note: T_1 : Sesame residue compost at 6 t ha^{-1} + RDF + Biofertilizers, T_2 : Wheat residue compost at 5.4 t ha^{-1} + RDF + Biofertilizers, T_3 : Pigeonpea residue compost at 4 t ha^{-1} + RDF + Biofertilizers, T_4 : Cotton residue compost at 7.1 t ha^{-1} + RDF + Biofertilizers, T_5 : Maize residue compost at 6.4 t ha^{-1} + RDF + Biofertilizers, T_6 : FYM at 6 t ha^{-1} + RDF + Biofertilizers, T_7 : FYM at 6 t ha^{-1} + RDF + without Biofertilizers, T_8 : RDF + Biofertilizers, T_9 : RDF alone

*Recommended dose of fertilizer is 25:50 kg N:P_2O_5 ha^{-1}.

Conclusion

Through the information generated from one year of study can be concluded that, the application of FYM, sesame, wheat, pigeonpea, cotton

and maize residue composts along with biofertilizers and RDF improved seed yield to the tune of 16.8, 14.66, 15.48, 15.11, 15.68 and 13.32 % over RDF alone. Residue-based composts applied on the N equivalent basis as that of FYM along with biofertilizers and RDF could be used as an alternative source to FYM for higher productivity of pigeonpea.

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Potential role of Azotobacter for improvement of soil quality and crop development: A Review

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ABSTRACT

Recently, biotic, and abiotic stresses are markedly influencing the growth and yield of the plant. In this juncture, the role of *Azotobacter spp.* in boosting plant growth and development is widely receiving popularity. *Azotobacter* stimulates the production of phytohormones such as Indole-3-Acetic Acid which act as a secondary messenger to withstand and

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resist several stresses. Moreover, it acts as a biofertilizer and makes the atmospheric nitrogen available to plants,and plays a key role in remediating soil contamination. However, the lack of adequate availability of commercial preparations of *Azotobacter* remains a major worry. The purpose of this review is to inform researchers about the functionality of *Azotobacter* spp. in improving plant wellness in a sustainable agricultural ecosystem. This article was framed with an aim of gathering a vivid knowledge about the potential role of Azotobacter for improvement of soil quality and crop development.

Keywords: *Azatobacter*, Nitrogen fixation, Phytohormones, Residue management, Crops, Performance

1. Introduction

Nowadays PGPR (Plant growth–promoting rhizobacteria) is increasingly being used in farming because it is an extremely useful tool for replacing chemical fertilizers, chemical pesticides, and other toxic elements (Gangopadhyay and Ghosh, 2019; Maitra *et al*., 2022; Mirriam*et al*., 2022). The action of these rhizosphere bacteria impacts directly or indirectly both the general morphology and physiology of the crops and produces massive amounts of growth-promoting chemicals. The latest advancements in sustainability are based upon the usage and diversity of Plant growth promoting rhizobacteria, their colonization potential, and their working mechanism which can be employed to make their deployment as a reliable thing in the managing of sustainability in farming systems easier. (Kumar *et al.,* 2015). Recent advances in applied research on Azotobacter species are of particular interest because they are both agriculturally significant plant growth promoting N2-Fixing rhizobacterium (PGPR) that can be used to improve plant N nutrition and a biofertilizer based product at large scale, having significant improvements in crop productivity and soil fertility. *Azotobacter* is aerobic nitrogen-fixing bacteria that comes under the free-living, gram-

negative bacterial group. They are oval or cylindrical and produce thick-walled cysts (they dormant their cells for resistance to harmful conditions) when exposed to unfavourable environments. About six*Azotobacter* species have been identified, out of which some are motile through flagella and others are immotile. (Sivasakthi *et al.,* 2017). In addition to BNF, Azotobacter species can directly affect plant development by synthesising plant growth hormones such Indole Acetic Acid (IAA), Gibberellins, and Cytokinins. These hormones can indirectly shield host plants from phytopathogens, promote other helpful rhizosphere bacteria, and improve plant growth and nutrient intake. Furthermore, numerous commercially relevant cereal and pulse crops showed considerable yield improvements thanks to the favourable effects of Azotobacter strains on plant development, crop output, and plant N needs (Aasfar *et al.,*2021). *Azotobacter chroococcum* has been proven to be important in crop yields for enhancing crop production and soil quality. These rhizobacteria synthesize amino acids that are involved in numerous activities, resulting in plant-growth promotion (Elshahat*et al.,* 2016).

Despite the fact that interest in beneficial Azotobacter species has slightly decreased over the past few decades due to thousands of research investigations on free N_2-Fixing bacteria in general and Azotobacter in particular, in addition to the agronomic potential of Azotobacter based biofertilizer species, their geographical distribution and diversity require additional specific studies.

2. *Azotobacter* as Plant growth promotors

The overuse of chemical fertilisers in agriculture causes the soil's fertility to decline and chemical pollution to rise. Therefore, it is crucial to implement modern technology that is economical, dependable, and eco-friendly. Azotobacter as a substitute for chemical fertiliser to boost soil fertility and crop yield in sustainable farming. The specific mechanism

behind the bacterium's growth-promoting effect is unknown. Various methods have already been hypothesized, including nitrogen-fixing, growth hormone synthesis, & siderophores production (Bano and Iqbal, 2016) .

2.1 *Azotobacter* as nitrogen fixers

Atmospheric Nitrogen fixation in the soil is vital for crops because, despite the fact that fresh air contains 78 percent of free nitrogen, plants cannot take it directly from the atmosphere. Biological nitrogen fixation is a key part of nitrogen equilibrium in the environment as it recycles nitrogen (Ghaly and Ramakrishnan, 2015). Additionally, microbial nitrogen fixation improves soil fertility maintenance and crop productivity improvement. *Azotobacter* has been proven to be beneficial as bioinoculants & also for monitoring the nitrogen fixation activity due to their capacity to proliferate quickly and fix huge amounts of nitrogen faster. *Azotobacter* can transform free nitrogen from the atmosphere into ammonia, which is then absorbed and used by crops. As the nitrogenase enzyme protects these bacteria from oxygen at the time of atmospheric nitrogen fixation, they are extremely resistant to it. There are additionally hydrogenase absorption& switch on off methods are there for protecting the nitrogenase enzyme from oxygen in addition to respiratory protection. (Ayuni *et al.,*2015). The ferrous (Fe) protein and dinitrogenase reductase are the two components of the nitrogenase enzyme. In a complicated process, those two chemical proteins catalyze the reduction of dinitrogen. (Verma et al., 2019). The existence of optimal calcium nutrient levels is important to *Azotobacter*'s improved development & ability of nitrogen fixation. On the other hand, high nitrogen amounts had a negative impact on the activities of *Azotobacter*. According to certain estimates, *Azotobacter*can fix approximately 20 kg of nitrogen per hectare annually, and therefore, can be used for crop production as a partial replacement for inorganic nitrogen fertilizers (Aasfar*et al.,* 2021). There

have been several reports of agricultural plants inoculated with *Azotobacter*requiring less nitrogen fertilizer. The use of a co-culture of *Azotobacter* strains might decrease the necessity of nitrogen fertilizers by up to half. Current advances in research activities involving *Azotobacter*spp. have attracted attention as an essential growth promoting and nitrogen fixing rhizobacterium (Plant growth-promoting rhizobacteria) as well as a biofertilizer product containing considerable benefits in crop production and improvement in soil quality (Bhat *et al.,* 2015).

2.2 *Azotobacter* for growth hormone production

Growth chemicals, commonly referred to as phytohormones, are natural molecules generated by both microbes and crops. Some physical and metabolic processes in microbes as well as plants are either stimulated or inhibited by them. *Azotobacter*spp. can effectively stimulate crop growth by producing plant growth hormones such as IAA, gibberellins, and cytokinin, in addition to biological nitrogen fixation.In vitro experiments revealed that when tryptophan is added to the media, *Azotobacter* produces indoleacetic acid. (Jiménez, 2005). Whereas just a few quantities of indol-3-acetic acid were found in ancient *Azotobacteria* culture that had not been supplemented with tryptophan. Additionally, gibberellin-like substances have been found in cultured *A. chroococcum*.In-vitro availability among these plant growth-stimulating gents is further supported by field trials with a variety of crops. The bacterium *Azotobacter* has been shown to produce certain growth hormones such as auxins and gibberellin-likecompounds, cytokinetic that have contributed to better crop production. These hormones emerge from the rhizosphere 0f the plant & have a favorable impact on the overall growth and health of bigger plants as well as small crops in the surrounding. When *Azotobacter chroococcum* was applied to diverse plants like tomatoes, corn, etc., the dry weight of seedlings was found much higher than when the plants were not inoculated (Nihorimbere*et al.,* 2012).

One of the significant potentials of how Azotobacter might encourage plant development is the solubilization of potassium (K) and zinc (Zn). The soil bacterium A. chroococcum has been shown by Wu et al. (2006) to improve the bioavailability of Zn in the soil system. This process involves a number of processes, including acidification. These soil-dwelling microorganisms secrete organic acids that bind Zn cations and lower the pH of the surrounding soil (Aung et al., 2020). Production of novel siderophores by A. chroococcum, such as vibrioferrin, amphibactins, and crochelins, which may bind iron in a hexadentate form utilising a new iron-chelating -amino acid, is another process that may be implicated in Zn solubilization. These siderophores not only aid in bacterial access to iron resources but also aid in plant management (Baars et al. 2015).

2.3 Synthesis of siderophores

Siderophores are a class of ferrous chelating compounds that change the distribution of Fe in the extracellular media by competing with other ligands. Siderophores are used by microbes to access vital iron minerals in the atmosphere. There are over 500+ siderophores identified, yet they only use a small number of basic moieties to retain ferrous. *Azotobacter* spp. use a Fe-siderophore complex to obtain access towards the sparingly soluble Iron within surrounding, which will then be taken via membranes attached receptor. Other competing microbes might not even have access to certain iron-siderophore complex, therefore they may have anti-phytopathogenic properties and that can significantly boost the growth of plants by providing protection from pathogen attack (Ahmed and Holmström, 2014). Further research has shown that the siderophores synthesized by *A. vinelandii* possess the capacity to binding metals apart from iron, therefore, allowing the absorption of other metals such as molybdenum or vanadium that are required in nitrogenase. This also includes harmful toxins such as zinc. Furthermore, *A. vinelandii* siderophores have been observed to

contribute to the growth of certain microalgae during co-culture, when a large amount of nitrogen is provided to such microbes. Baars discovered over 35 metal-ligand secondary metabolites in the siderophore metabolome of *A. vinelandii* in 2016, pointing to the huge chelome of *A. vinelandii*, which also included vibrioferrin, which was initially believed to be in oceanic bacteria. In addition to *vibrioferrin*andamphibactins, *A. chroococcum* has been observed to manufacture the crochelins, which is a side resource. Despite its agricultural importance, the second metabolome of *A. Chroococcum* was unknown. Moreover, structures of siderophores andthe process through which *A. chroococcum* gets access to iron that is required to create high levels of nitrogenases have still not been calculated (Baars *et al.,* 2018).

3. *Azotobacter* used for bioremediation

Bioremediation is the process of removing pollutants, wastes, & poisons from soils, waterbodies, and other parts of environments by using living microbes such as microorganisms. Microbes are being used as a food supply in microbial bioremediation to dissolve pollutants. Bioremediation is a good way to reduce environmental pollution. The stimulation of natural soil microbes that can eat toxins or even the introduction of effective isolation of microbes to polluted soil are the two most used bioremediation strategies. Free-living *Azotobacter* takes part in nitrogen fixation andmakes up a significant percentage of the soil microflora (Sivasakthi *et al.,* 2017).

3.1 *Azotobacter* in Pesticide degradation

In polluted soil, microbes are an excellent source of pesticide solubilization.Lindane, commonly called hexachlorocyclohexane, is a wide-spectrum organo-chlorine pesticide that is widely used across India.It's been suggested that it could cause cancer (Zhang *et al.,* 2020). Microbes can utilize pesticides given to soil and thus they can degrade

them. *Azotobacter*'s capacity to utilize aromatic chemicals has been recognized for a long time. It can decompose aromatic chemical derivatives such as p-hydroxybenzoate, protocatechuic acid, benzoate 2,4-D,2,4,6-trichlorophenol, and others. Some other chlorinated phenols are also degraded by *Azotobacter* such as 4-Chlorophenol, 2-Chlorophenol, 2,6-Dichlorophenol. As a single source of carbon, *A. chroococcum* extensively metabolized 2,4-dichlorophenoxyacetic acid. In situ and Ex situ at lower concentrations like 10 ppm, some strains of *A. chroococcum* were demonstrated to be successful in pesticide breakdown. Although, bacteria's ability to break down lindane is found to be diminished with greater concentrations. This could be because lindane has an inhibiting effect on microbial growth at greater concentrations. *A. chroococcum* has been shown to be capable of converting a common herbicide, pendimethalin, into non-toxic compounds, demonstrating that the bacteria is important not only just for crop development but for environment harmony (Abdallah *et al.,* 2021).

3.2*Azotobacter* in the eradication of oil-contamination

Bacteria belonging to the *Azotobacter* genus have been found to use a wide variety of organic substrates as a source of carbon and energy, including various organic acids, mannitol, phenolic compounds from soil, and others, to produce several biologically active compounds that promote rhizospheric microbe propagation. As a result, it is reasonable to conclude that these microorganisms could contribute to the bioremediation of oil-polluted soils (Rubio ***et al.,*** 2013). As *Azotobacter* can assimilate oil hydrocarbon molecules in the existence of fixed nitrogen as well as during the fixing of nitrogen, it can speed up the pace of purification of oil-polluted soil. *Azotobacter chroococcum* has been known to enhance the growth of hydrocarbon oxidizing microbes in microbial preparations such as Devor oil. The results of environment-friendly bioremediation of olive oil mill effluent by *Azotobacter vinelandii*

proved *A. vinelandii*'s ability to multiply in olive oil mill wastewater utilizing its own ingredients, converting olive oil mill wastewater into organic liquid fertilizer. Similarly, this technique eradicated the phytotoxic components from olive oil mill wastewater, as well as the microbial populations that are important for agriculture (Mekki ***et al.,*** 2013).

3.3 *Azotobacter* in tolerance of heavy metal

The soil microbe population is under great stress because of soil impurities by a variety of hazardous compounds, which includes heavy metals and other organic pollutants from polluted water, industrial wastewater, and other pollutant sources. Heavy metals in various forms introduced into the atmosphere cause considerable changes in microbial biodiversity and activity, influencing soil quality directly (Wuana and Okieimen, 2011). Under extremely reduced concentrations inside the cell, certain heavy metals are required for microbe growth and metabolic activities. Although, as heavy metal concentrations rise, they become increasingly hazardous to microorganisms, disrupting essential natural processes. Environmental heavy metal pollution had contributed to the growth of heavy metal resistant bacteria in metal-polluted soils. Furthermore, once in the soil, heavy metals collect selectively in the areas where crop roots accumulate and in forms that are readily accessible to plants. Those heavy metal ions are subsequently taken by plants, eventually making their way into the food chain. Microbes utilize a variety of strategies for resistance to heavy metalsand detoxifying it. As a result, they play an important role in the biogeochemical cycle of toxic heavy metals, which leads to the treatment of metal-polluted habitats. Ten strains of *Azotobacter* were isolated from soil that is polluted with wastewater and showed significant tolerance to heavy metals such as cobalt ion, nickel ion, zinc ion, and copper ion, etc. As a result, the research raised the possibility of using such isolated bacteria for bioremediation of systems contaminated with metal. The current study discovered that heavy metal tolerant strains of *Azotobacter* have

a strong potential to bind with cadmium andchromium, and thus exert considerable control over their absorption by wheat plants grown in heavy metal contaminated soils. Plasmids are shown to induce tolerance towards heavy metals in *Azotobacter* species. However, before the introduction of heavy metals into the cell, *Azotobacter spp*. is confronted with extracellular polymeric compounds, which also are claimed to be generated in significant quantities by such bacteria. Withthe ability to bind metal ions and prevent their entry into the cells of bacteria, extracellular polymeric molecules simply serve as thefirst shield (Asati*et al.,* 2016).

3.4 *Azotobacter* in the salty environment

Salinity is the primary non-biotic stress factor that affects plant growth and development among the different non-biotic stresses. Salt content disrupts the fluids and ionic flow of plant cells, affecting plant growth, development, structure, biology, and many activities, ultimately resulting to plant mortality. Soil salinization is caused by a variety of activities, including anthropogenic operations, but the principal source is natural processes, which result in significant salt buildup in surface and underground water (Kumar, 2020). Beneficial bacteria may be the agent that may be used in this situation. They influence crop growth and biochemical indicators in addition to helping to produce biological substances that provide defence against non-biotic stresses (Ahmad et al., 2021). Furthermore, it has previously been established that PGPBB increases crop development by lowering both biotic and abiotic stresses. It is believed that a number of processes that influence crop growth and production capability take place at the soil-microbe interface. It is believed that the application of beneficial microorganisms that promote plant development is especially crucial for extending crop life by reducing stresses under unfavourable conditions. In modern sustainable agricultural farming, nitrogen-fixing bacterial strains of the Azotobacter species are used extensively and successfully. Azotobacter species are

renowned for fixing atmospheric nitrogen, producing siderophores, indole-3-acetic acid, and synthesising exopolysaccharides, all of which are beneficial to crop health (Kumar and Verma, 2019).

4. *Azotobacter* role in plant disease management

Azotobacter has been linked to the prevention of plant disease illnesses, apart from its favorable effect on crop development and health.There ismuch evidence in the research that shows the relevance of pathogen control by various *Azotobacter* spp. Maheshwari in the year 2012 found that *A. chroococcum*'s TRA2 strains, that is a wheat rhizosphere isolate, had a considerable inhibitory effect versus the root rot disease caused by root rot fungi *Fusariumoxysporum* and *Macrophominaphaseolina* , and also improved wheat plant development, perhaps because of improved crop health (Gulab *et al.,* 2016).*Azotobacter* provides excellent crop defense by invading wheat crop roots strongly.The root-knot illness caused byMeloidogyne incognita was greatly decreased whenever *A. chroococcum* has been administered to chickpea.The control tactics utilized by microorganisms to prevent crop pathogens can be associated with several factors.Siderophores, antibacterial compounds, poisons, and growth stimulants such as auxins, cytokinins, and gibberellins are examples of these. Although, there is no specific process that can be considered entirely accountable in disease prevention, because bacteria may utilize many mechanisms according to the strain of bacteria, ecological factors, microorganism involved, & target organism.Such bacterial techniques were shown to offer significant tolerance to crop disease assault.Again, further research showed that only 37percent of the entire strains were capable to prevent *Rhizoctoniasolani* from growing and around 25percent were capable to prevent *Xanthomonas campestris* from growing. However, the nature of antibacterial compounds indicated that most antibacterial molecules were extracellular, just with a few attached to the cell wall.*Azotobacter* had the potential in synthesizing siderophores which can attach to the free iron present

in the rhizobia, restricting phytopathogens' access to iron thereby maintaining good plant health.*Azotobacter* spp. has been found to develop an antibacterial substance with a structure comparable to neomycin, a well-known fungicidal agent.*Alternaria, Rhizoctonia, Curvularia, Macrophomina, Helminthosporium,* etc. are some of the diseases that have been controlled using *Azotobacter* as an ecofriendly microorganism (Gunardi, *et al.,* 2021).

5. Recent trends of application of *Azotobacter* as abiofertilizer

Because *Azotobacter*is a non-symbiotic bacterium, its whole capacity for increasing crop output can be realized by combining it with different biofertilizers rather than using it alone.*Azotobacter*, in addition to actively benefiting crops by increased mineral absorption, also accelerates the helpful effects of various biofertilizers when applied in combination with other biofertilizers.There are examples of some other bacteria improving *Azotobacter*'s crop-yielding activities.Nowadays, many examples of *Azotobacter* getting used in association with various bacteria are gaining popularity among researchers and farmers (Jnawali*et al.,* 2015).

5.1 *Azotobacter* along withanother biocontrol fungus

Among various fungi biofertilizers, phosphorus solubilizing mycorrhizal fungus have been found to work well with *Azotobacter* to improve crop development. Many researchers have observed a beneficial interaction among the nitrogen fixation by living organisms, Azotobacter bacterium and the AM fungi *Glomus* (MeCarty***et al.,*** 2017). El-Shanshoury et al. (1989) found that, in comparison to crops treated with either *Glomus fasciculatum* or *Azotobacterchroococcum* separately, the greatest densities of bacteria found from the rhizosphere from tomatoes treated with the both *Azotobacterchroococcum* and *Glomusfasciculatum*.*G. fasciculatum* treatment of tomatoes increased *A. chroococcum* number throughout the rhizosphere, which was retained

at a higher level for an extended duration.Moreover, Treatment of tomato roots using *A. chroococcum* promoted *G. fasciculatum* infections and spore formation.As compared to untreated crops, the average dry weight of tomatoes treated using both *G. fasciculatum*and*A. chroococcum* has been shown to be considerably higher. In wheat crops, Bona *et al.*(2015) foundthat double treatment of AM fungi along with *Azotobacte*r had equivalent results. Wani*et al.*(2013) found that, When Punica granatum crops were treated using a blend of *A. chroococcum* and *Glomus mosseae*, they were capable of surviving duringunfavorable environmental conditions also. According to Kilam et al. (2018), the arbuscular mycorrhiza fungus *P.indica* and bacterium *Azotobacterchroococcum* created a mutually beneficial symbiosis in sweet wormwood, which resulted in enhanced crop biochemical and physiological properties, as well as increased artemisinin concentration**.**

5.2. *Azotobacter* in a bacterial consortium

Effective results found from plants treated together using *Azotobacter*and*Rhizobium* have been observed in the laboratories, greenhouses, and fields. *Azotobacter* can synthesize growth hormones such as auxins, gibberellins, etc. which stimulate the growth of roots and thus provide more root surface exposed to rhizobacteria for infections. Because of this higher nodulation, nitrogen-fixing, plus improved agricultural yields will be the outcome (Tiwari *et al.,* 2017 discovered a combined impact of *A. chroococcum* with *Bradyrhizobium*on *Vigna radiata* crop, while Ara et al. (2009) found the same on chickpeas. *Azospirillum* is also a bacteriumthat has been linked to *Azotobacter* in a mutually beneficial relationship. The effects of *Azotobacter* Plus *Azospirillum* treatment together on the yields of chickpea have been reported to be effective (Rueda *et al.,* 2016). Symbiotic treatments of*Azospirillum* and *Azotobacter* have been reported to relieve the negative effects of salinity stress on various plants, in addition to enhancing crop yields and yield qualities. According to, Seeds of a Hop

Bush, treated using *Azospirillum*bacteria along with *Azotobacter*bacteria when disclose into salty conditions demonstrated better germination percentage and crop growth characteristics. The benefits of co-treatment of *Azotobacter* along with *Azospirillum* to crops are primarily determined by their ability to enhance root growth, speed of water as well, and to a lesser extent, alleviation of non-bioticstressors on crops (Reddy *et al.,* 2018).

6. Molecular approaches which can enhance *Azotobacter*'s biofertilization characteristics

It has been suggested to use *Azotobacter* species as organic fertilizer in order to restore nitrogen levels (Dikr and Belete, 2017). When working to improve the nutritious characteristics of *Azotobacter* as a biofertilizer, it is important to think about cost-effective strategies which can give a reduced-priced source of biofertilizers to the agricultural sector. While considered huge-scale*Azotobacter* manufacturing, it is important to optimize biological and nutrition parameters to improve its fermenting capacity as well as its biofertilizers capacity. Biotechnological and industrial interest in microbial treatment andpolymers formed from them has grown because of its helpful features andtheir potential to create newer chemicals that could be used as an efficient tonic in soil & crop health care. *A. vinelandii* is very beneficial in biotechnological applications because of its capacity to create key biotic compounds such as PHB, exopolysaccharides, & siderophores. (Poli *et al*., 2011). The nitrogen-fixing activity of *A. vinelandii* could b greatly improved by using genetic modifications to introduce and delete a specified gene. The ammonia of ordinary compounds is transformed to end products because of the specific gene alteration. Causing disruption of the nifL genes from the nifLA operon network could be used to increase the ammonia concentrations excreted by *A. vinelandii*. Moreover, the soil's ecological properties of various places vary significantly. This is the reason why Only one strain of *Azotobacter* can be successful in all places and hence,

will be used as a biofertilizer worldwide. Considering the usefulness of EPS and similar molecules formed in the development of the bacteria in farm soils in consideration, *Azotobacter* strains with characteristics such as the maximum capacity of nitrogen fixation and better production of these molecules must be considered. Additionally, future studies to improve our knowledge of such qualities by modifying them to fulfill the requirements of humans could be a major factor in the farming of future generations (Sivasakthi *et al*., 2017).

Azotobacter is among the finest selections for using it as a biofertilizer for sustainable agricultural production because of its capacity to increase crop growth via nitrogen - fixing, growth hormone secretion, solubilization of phosphorous, prevention of plant disease, as well as remediation of improved soil quality (Bhardwaj *et al*., 2014). Identifying and managing all*Azotobacter*'s favourable traits could be a crucial goal for further crop development efforts. Further research on refining screening approaches, isolating and characterization of crop growth stimulating and antibacterial substances from isolated bacteria, and elucidation of the molecular scale of processes linked are all needed. Finding appropriate partners, i.e., a specific strain of *Azotobacter* that would create a good symbiotic relationship with certain crop genotypes is a task for the scientific society to ensure optimum advantage from the biofertilizer (Namvar and Khandan, 2013).

8. Conclusion

The use of Azotobacter spp. can be quite helpful in removing different stressors. To enhance the soil's physical and chemical qualities, potential strains are also introduced. In the presence of the appropriate strains, the microbiome of the rhizosphere is also altered, which is thought to be particularly advantageous for the enhancement of plant health. It has been argued and supported that the use of Azotobacter spp. in different field crops has eliminated plant stresses of diverse origins.

The manufacturing of several beneficial organic compounds has been accelerated in plant tissue, strengthening the plants and enhancing their ability to fend off stresses. To fully understand the methods by which Azotobacter spp. eliminate stresses and improve plant health, substantial study is still required. In a nutshell, Azotobacter spp. might reduce the stressors placed on a variety of agricultural crops as a result of biotic and abiotic factors.

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Influence of Soil Rhizobacteria for Improving Drought Resilience in Legumes: A Review

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ABSTRACT

Legumes play an important role in feeding the growing population by becoming the medium for high-quality protein at a cheap price. Legumes improve the circulation of nutrients in the soil and increase the retention of water in the soil. The soil rhizobacteria help the legumes to maintain the soil health and increase the number of microbes resulting in their

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diversification. Due to these reasons, legumes can become a source to reduce the dependence on chemical fertilizers and pesticides. The only drawbacks in cultivating legumes are the abiotic stresses that may affect their growth and productivity. So, for the cultivation of legumes, we need to develop some varieties of rhizobacteria that are resistant to abiotic stresses. In this view, this review highlighted the need of harnessing soil rhizobacteria for improving drought resilience in legumes.

Keywords: Legume, Rhiobacteria, Drought resilience, Soil microbes

Introduction

The human population in recent years has been increasing significantly at a rate of 1.05% per year. An increase in the population comes with new needs and challenges like enough amount of food with proper nutrition, a suitable and pollution-freeenvironment, etc. Among all types of crops, legumes have significance and have been valued as a sustainable and cost-effective food grain that withstand unfavourable conditions resulting to build up food security (Maphosa*et al.*, 2017; Jena *et al.*, 2022; Mirriam*et al.*, 2022). In addition, the leguminous crops facilitate nutrient circulation in the soil, water retention; increase the organic matter content in the soil, help in the diversification of microbes present in the soil, etc. Without a doubt, chemical-based agricultural intensification has changed agriculture by considerably boosting global food production. A review of the effects finds that they have had a disproportionately large influence, notably in underdeveloped low-input systems (Carvalh o*et al.*, 2017). Chemical fertilisers may have improved soil fertility while adversely affecting its biology. In addition to the environmental and energy cost of producing chemical fertilisers, the reduction in soil health and microbial populations, as well as negative changes in the structure of the soil, have been highlighted as key side effects of increased chemical use. So, increasing agricultural productivity without forcing any kind of stress or bad impact on the environment

and maintaining food quality with minimum expenditure has set off a new challenge in the modern world. As a result, it is now becoming more widely recognised that soil biology should be restored by using soil microorganisms and legume relationships. In addition to promoting development, the apparent advantages also include a rise in system resilience. Given that grain legumes are almost always cultivated in low-input marginal agricultural systems with little assistance and resource limitations, especially water, this is all the more crucial. Because grain legumes have an evolutionary history of coexisting with bacteria, it is essential to promote health in order to maximise the advantages of microorganisms. The potential contribution of soil rhizobacteria to the enhancement of the resilience and sustainability of farming systems based on legumes will be covered in this chapter.

Importance of legumes

Legumes are essential and sustainable crops either as the main growing crop or as a crop residue. In the year 2018, the world produced about 92.4 million tonnes of pulses. According to FAO, India has the world's largest area for leguminous crops, at about 29 million hectares, with a production of 22-24 million tonnes (Oyewale*et al.,* 2013). The collaboration among ConsultativeGroup on International Agricultural Research (CGIAR) institutions, like ICRISAT(International Crops Research Institute for the Semi-Arid Tropics), ICARDA(International Center for Agricultural Research in the Dry Areas) and CIAT(International Center for Tropical Agriculture) and national agricultural research institutions across the world has resulted in the development of new varieties that can survive under stressful environmental conditions without affecting the yielding capacity and nutritional quality of the crop(Plucknett*et al.,* 2020). Legumes have an important role in improving the indigenous soil nitrogen content and soil quality besides meeting the demands for protein and energy.

Consequence of Drought Stress in Legumes

Abiotic stress can causea reduction in the growth of plants leading to a reduction in the yield. Among these, drought due to the unavailability of water is a primary cause of crop loss worldwide which occurs frequently. This abiotic stress when comes along with other stresses like elevated temperature becomes more negative(Balfagónet *al.,* 2020.). Legume crops are naturally sensitive to conditions like drought because it affects the formation and longevity of the root nodules. Water stress in the early stages of the vegetative stage has less effect as compared to that of flowering or the pod-filling stage of the crop. Among legume crops, field pea, lentil, groundnut, soybean and pigeon pea have resistance to water stress, thus they sufferrelatively lesser yield reduction under drought as compared to faba bean, chickpea, green gram and cowpea(Smykal, *et al.,* 2015). Crops that are least resistant to water stress are Bambara bean, lablab bean, common bean and black gram. With the population expanding continuously, the increase in demand for pulses, the shortage of land and irrigation facilities for agriculture has made it difficult for the cultivation of leguminous crops (Vurayai*et al.*, 2011).

Impact of Water Stress During N-fixation in Legumes

Rhizobium present in the root nodules of the legumes is responsible for the biological N-fixation which is a prime contributor to boosting the fertility of the soil. As the N-fixation is susceptible to water stress, thus the plant will suffer from nitrogen deficiency that will further reduce its nutritional quality (Singh*et al.,* 2021). Therefore, it is concluded that the Rhizobium in the root nodules is more sensitive to water stress.

Legumes -Rhizobacteriaassociation

In 1904, Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research,coined the term "rhizosphere" to describe a

part of the soil present around the roots of legumes that is heavily under the influence of microbes. The rhizosphere is more eminent than the edaphosphere, with a well built up microbial activities and an increased amount ofroot exudates(Nihorimbere*et al.*, 2011).In legumes, the microbes generally bacteria form a symbiotic relationship with the plant roots which leads tothe formation ofnodules(Zgadzaj*et al.,*2016). The genera of bacteria that can colonize with the roots of the legumes and show significant effects on the plant growth are known as plant growth-promoting rhizobacteria (PGPR). PGPR enhances the growth in the legumes by increasing their nutrient content, regulating the hormone and enzyme levels, or by developing the biocontrol agent forms and decreasing the inhibitory effects of different pathogens attacking the plant(Gupta*et al.,* 2015).There are different types of plant-microbe interactions which can take place in the soil depending upon the contiguity of the roots of legumes with the rhizosphere, among which endophytic interaction is one.This interaction helps in preparing the legume crop against biotic and abiotic stresses and thereby enhancing growth and yield(Saidi*et al.,* 2021).

Soil Rhizobacteria Amelioratethe Drought Stress

Crop plants may experience different kinds of stress like water stress, alkaline stress, stress due to high temperature, etc that can act as limiting factors and leads to several damages. PGPRinteracting with legumes crops helps in improving the production, quality and capability to recover from different stresses(Ilangumaran*et al.,* 2017). For example, Rhizobium synthesises a sugar known as trehalose in the nodules of the plant that helps to retain water in the cells(Tookmanian*et al.,* 2021).

Water stress in legumes leads to several complications in the formation and functioning of nodules, biosynthesis of protein, leghaemoglobin, etc.(Stambulska*et al.,* 2018).These stressful conditions can be mitigated by rhizobacteria through the following mechanisms:-

a. During the stress conditions, stimulation of the activity of phytohormones like ACC deaminase by the production of IAA by bacteria(Khan*et al.,* 2016.). IAA willenhance the production of ABA whichwill regulate the water fluxes.

b. ACC deaminase enzyme reducesthe production of stress-induced ethylene during stress conditions(Barnawal*et al.,* 2014).ACC-deaminase is sensitive to water stress in the roots and leaves.

c. In the root tissue, active forms of plant growth-promoting compounds are released through hydrolysis of conjugated phytohormones and flavonoids.

d. PGPRs in Arabidopsis can delay reproduction to tolerate the water stress.

e. Triggering the induced systemic resistance using the bacterially derived biofilms, i.e. extracellular matrix can also help in controlling water stress.

Rhizobacteria helps in favourably modulating the plant-centric reaction of the host plant to stresses. These microbes are very sustainable in both economical and ecological aspect (Turan e*t al.*, 2014).

Breeding Perspective of Harnessing Soil Rhizobacteria

Some other helper microbes work with the crop plant along with rhizobacteria. Their level is determined based on the role they play in managing different stresses (Elshahat*et al.,* 2016). There are certain combinations of microbe groups that produce a maximum effect when working together. Therefore, the selection of suitable combinations is also important.

Selection of Differential Genotypic Response to Rhizobacterial Inoculation

The crop microbial associations that are beneficial can be selected by following some steps. First, proper knowledge about the legume crops, its varieties, and their breeding lines is required. The knowledge about the association of germplasm with different rhizobacteria and the identification of genotypes suitable are also important (Amaral *et al.*, 2021). These processes are gradual processes as varieties of conditions can occur while selecting the suitable legume crop and microbe. For example, legumes having genotypes that can maintain higher levels of nitrogen fixation and concentration of nitrogen in the product are selected(Clua*et al.,* 2018). Wild varieties of legumes are given more importance as they can provide valuable genes that can help to tackle stress condition.

Selection of Competitive Rhizobacterial Strains

The rhizobacterial strains that have undergone many evolutionary changes or mutations are preferred due to their ability to survive in stressful conditions. The ability of rhizobacteria to develop a symbiotic relationship with the legumes depends upon the change in traits of the legume plant after the rhizobacteria was attached to it(Alemneh*et al.,* 2020). They should be able to produce oxygen in stressful conditions, adaptive in nature, larger nodulation with high nitrogenase activity. The nod gene expression in rhizobium plays a key role in its symbiotic relationship with legumes. Nod genes in the rhizobium plays an important role in attracting beneficial bacteria towards the plant root for inducing nod factor production(Khan*et al.,* 2012). These are regulated by the flavonoids released by the legumes along with the positive activator NodD(Liu*et al.,* 2016). The desired rhizobacterial strains should be able to overpower the native microbial colonies so that, it can establish a good plant-microbial symbiotic relationship. After

the desirable genetic characteristics are short-listed, these genes are introduced in the commercial rhizobacterial strains and their behaviours are analysed in the natural habitat to identify any kind of complications (Debasis *et al.*, 2020).

Identification of Adaptive Crop-microbial Associations

The selection of good genes and introduction of them to the strains of rhizobacteria are not enough to identify and breed a fine-quality rhizobacterium. It must be experimented on the fields, with exposure to the environment with stresses to analyse results. An important character to be considered is the root phenes (Bailey, 2018).Root phenes (like root growth rate, branching of lateral roots, root hair length, density of root hairs, etc.) constitute the phenotype of the roots and are responsible for the accumulation, mobility and utilization of resources(Lynch*et al.,* 2014).Apart from the genes of the rhizobacteria, the soil should also be in appropriate conditions.The soil with low pH, acidic nature, and high H+ concentration affects the plant and also the bacteria in the soil inhibiting their growth. Therefore, strains of rhizobacteria that can handle low pH, acidic conditions should be isolated and developed as about 40% of the world's land are acidic in nature(Saikia*et al.,* 2018).

Future Perspectives and Conclusion

In the world of surging population, the call for abundant of nutritive food without appropriate climatic conditions has become a massive challenge for farmers as well as scientists.Among all the crops, legumes will have a prime importance to meet the food and nutritional insecurities of the world. The diversification of crops, isolation of genetically modified varieties of crop plants as well as soil living microbes will encourage the farmers to minimize their reliance on chemical fertilizers. This will also ensure and improve the soil quality and will bring down the adverse effects on the environment. This will also

gradually aid the natural potential of the plants to survive under stressful conditions, and their immunity against pests and diseases. In order to comprehend the routes employed by rhizospheric microorganisms in induced systemic tolerance and rhizospheric engineering under drought stress, further molecular research on plant-microbe interactions will likely be required in the future. Therefore, subsequent studies should concentrate on bio formulations, such as strain encapsulation, to guarantee the effectiveness of bio-inoculants in real-world settings. The ultimate objective of this method is to increase the ability of plants to withstand drought while also promoting the colonisation and spread of helpful bacteria. A potential strategy to lessen the negative effects of climate change on agricultural yield and sustainability is to better understand the interactions between plants, related microbes, and the environment.

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Recent Developments in the Control of Ralstonia Solanacearum Infestation in Eggplant

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Abstract

Ralstonia solanacearum is a major phytopathogen that attacks a wide range of crops and other plants. The extensive genetic diversity of strains responsible for various bacterial wilt diseases has led to the concept of an *R. solanacearum* species complex in recent years. The progress made in producing eggplant cultivars or hybrids resistant to bacterial wilt is encouraging. However, because the pathogen is developing rapidly, it is critical to understand pathogen dynamics and

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develop effective screening procedures. Breeders must understand the genetic variety of eggplants and their wild relatives, gene activity, and available molecular markers. Grafting technology, in which resistant sources are employed as rootstocks, is also gaining popularity. This method saves time by avoiding the work required to change genetic backgrounds through breeding. The focus of this review was on current improvements in control measures such as biological, physical, chemical, and cultural measures, as well as biocontrol efficacy and suppression mechanisms.

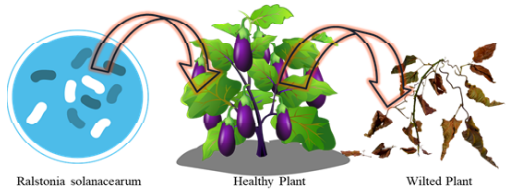
Keywords: *Ralstonia solanacearum,* Bacterial wilt, Eggplant, BCA, Plant Breeding.

Introduction

Eggplant (*Solanum melongena* L.) often known as brinjal or aubergine and dubbed "the poor man's vegetable," is a widely farmed solanaceous vegetable crop. Globally, 54.1 million tonnes of brinjal are produced from 1.86 million hectares of land, with a productivity of 29.1 kg per hectare (Food and Agriculture Organisation statistic, 2018). China (56% of global output), India (26% of global output), Egypt, and Turkey are the largest brinjal producers. This infection is a severe concern in sections of Kerala, Orissa, Karnataka, Maharashtra, Madhya Pradesh, and West Bengal in India. (Rao et al., 1976) The fruits are a good source of dietary fibre, calcium, proteins, phosphorus, iron, vitamin A, vitamin B, and vitamin C. Because the fruit contains a high percentage (65%) of polyunsaturated fatty acids, magnesium, and potassium, it serves as a cholesterol-lowering agent and is used as a treatment to control high blood cholesterol and liver disorders. (Daunay and Hazra, 2012) Skin illnesses, coughs, toothaches, piles, inflammation, throat troubles, and stomach problems can all be treated using eggplant root and leaf extracts. (Mak, 2013)

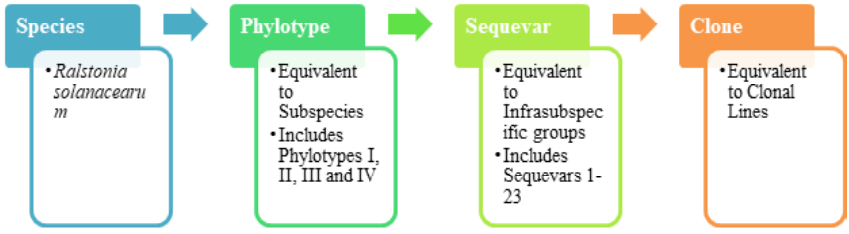
R. solanacearum, a â-proteobacterium, is pathogenic to over 200 plant species from over 50 botanical families. The pathogen not only affects solanaceous plants like tomato and potato, but also many weeds, crops, shrubs, and trees from other dicot and monocot families. (Denny, 2006) *R. Solanacearum* can survive in moist soils or water microcosms for years. When a pathogen comes into contact with a susceptible host, it enters the root and colonises the root cortex before invading the xylem vessels and quickly spreading to aerial parts of the plant via the vascular system. The vascular dysfunction caused by this extensive colonisation causes wilting symptoms. (Alvarez et al., 2008) *R. solanacearum*'s direct yield losses vary greatly depending on the host, cultivar, climate, soil type, cropping pattern, and strain. For example, tomato yield losses range from 0 to 91%, potato yield losses from 33 to 90%, tobacco yield losses from 10 to 30%, banana yield losses from 80 to 100%, and groundnut yield losses from up to 20%. (Elphinstone, 2005)

Disease management through proper farming practices requires continued development and adaptation. As a result, cultivating resistant variants could be an effective solution to the problem. Growing disease-resistant brinjal is a long-term, environmentally benign, and successful disease control method. Crop improvement programmes will be successful if various genetic sources are available and accessible. Unfortunately, the resistance response of commercial cultivars is known to fluctuate both geographically and over time due to genetic heterogeneity of pathogen strains as well as environmental differences. Because there is no one way for completely eliminating bacterial wilt, farmers will benefit from integrated crop management practises and generally accepted disease resistance types.



Pathogenicity of *R. solancearum*

Understanding the diversity of *R. solanacearum* strains is useful for identifying subspecific groups of strains with shared geographical origins, biological features, and evolutionary relationships. (Barik et al., 2020) *R. solanacearum* species complex (RSSC) strains are classified into five phylotypes: phylotype I from Asia, phylotype IIA from northern Latin America and the Caribbean, phylotype IIB from South America, phylotype III from Africa, and phylotype IV from Indonesia, Australia, and Japan. (Fegan and Prior, 2005) *R. Solanacearum* is divided into two groups based on RFLP (restriction fragment length polymorphism) fingerprinting: Division I originated in Asia (biovars 3, 4, and 5) and Division II originating in South America (biovars 1, 2 A, and 2T). (Hayward, 1964) This knowledge will aid in the development of breeding methods for long-term resistance to bacterial wilt, as well as the identification of different R. solanacearum strains connected to pathogenicity for specific hosts. (Barik et al., 2020)



R. solanacearum can be found in all sorts of soils, but prefers acidic soils and coastal wet environments. It is typically found in tropical and sub-tropical countries, and it multiplies and spreads more rapidly in hot and humid climates. The pathogen enters the plant through wounds or secondary root initiation points, colonising vascular parenchyma and causing cell wall breakdown. (Vasse et al., 1995) Rapid multiplication of

R. solanacearum occurs in the xylem, causing water transfer to be blocked and plants to wilt, eventually leading to death. The first signs of wilt are leaf drooping, which is followed by whole plant withering and vascular discolouration. It spreads to neighboring plants through root contact, contact with a water supply, or contact with humans or machinery. Milky white ooze can be seen when the cut ends of the wilted plant stem or root are immersed in water. (Barik et al., 2020)

Phylotype	Origin
I	ASIA
IIA	AMERICAs
IIA^T	AMERICAs
IIB	AMERICAs
III	AFRICA
IV	INDONESIA

Race	Geographical Distribution
Race 1	The world's tropical regions.
Race 2	Tropical areas of Central and South America, as well as Asia (Philippines, Indonesia).
Race 3	Tropical, subtropical, and temperate high-altitude environments.
Race 4	Asia (Philippines)
Race 5	Asia (China)

Methods implemented for protection of Eggplant against Bacterial wilt

Chemical methods

Pesticides have been significantly responsible for plant disease control. To control bacterial wilt, pesticides such as algicide (3-[3-indolyl]

butanoic acid), fumigants (metam sodium, 1,3-dichloropropene, and chloropicrin), and plant activators (validamycin A and validoxylamine) have been utilised. The combination of methyl bromide, 1,3-dichloropropene, or metam sodium with chloropicrin reduced bacterial wilt in the field upto 72%. (Fortnum and Martin, 1998, Kiely et al., 2004, Santos et al., 2006) Pesticides provide more benefits than alternative control methods; but, if farmers use pesticides carelessly or without sufficient information, a portion of the pesticide may persist in the environment for many years, becoming a pollutant in soil and/or groundwater and harmful to farmers. (Dasgupta et al., 2007)

Biological methods

Concerns about the widespread use of chemicals have boosted interest in biological control. Biological control agents (BCAs) have the following advantages: 1) they are potentially self-sustaining, 2) they expand on their own after initial establishment, 3) they require less nonrenewable resources, and 4) they suppress illness in an environmentally benign manner. BCAs' processes are supported by interactions like as competition for nutrients and space, antibiosis, parasitism, and induced systemic resistance. (Ramesh and Phadke 2012)

Organic soil supplements have a direct effect on plant health and agricultural output. Organic matter decomposition in soil can directly affect pathogen viability and survival by limiting available nutrients and releasing natural chemical compounds with different inhibitory characteristics. Organic soil additions have been found to boost the activity of pathogen-fighting microorganisms. (Bailey and Lazarovits 2003)

Physical methods

A variety of physical control measures, such as solarization and hot water treatments, have been shown to be effective against R. solanacearum. Soil solarization decreases soil pH, potassium (K), sodium

(Na), boron (B), and zinc concentration, microbial biomass, and microbial respiration, but has no effect on other soil chemical characteristics. Prior to planting, the infected soil should be heated at 45°C for two days or at a minimum temperature of 60°C for two hours. Before the application of soil solarization may be broadened, several aspects must be carefully considered: managing temperature or the release of volatile compounds, as well as financial and/or practical viability in the field. Cold temperatures, in addition to heat therapies, can be useful in some cases. (Kongkiattikajorn and Thepa, 2007)

Cultural practices

The development of bacterial wilt-resistant cultivars is regarded as the most cost-efficient, ecologically friendly, and successful means of disease control. The availability of resistance sources, their diversity, the genetic linkage between resistance and other agronomic traits, differentiation and variability in pathogenic strains, the mechanism of plant-pathogen interactions, and breeding or selection methodology have all been major factors in bacterial wilt resistance breeding. (Elphinstone, 2005, Boshou, 2005)

Crop rotation benefits include soil structure and organic matter preservation, as well as a reduction in soil erosion, which is commonly associated with continuous row crops. While continued farming with the same susceptible host plant will result in the establishment of certain plant pathogenic populations, crop rotation avoids this negative effect and is frequently related with a decrease in plant illnesses caused by soilborne pathogens. (Janvier et al., 2007)

Previous research found that fertiliser treatment reduced the occurrence of bacterial wilt. Calcium (Ca) is the most well-known disease suppressant fertiliser. The use of rock dust in conjunction with commercial organic fertiliser reduced the occurrence of bacterial wilt. whereas greater soil pH and Ca content were important contributors

in the rock dust amendment's prevention of bacterial wilt. (Li and Dong, 2013)

By avoiding time-consuming resistance screening and saving time in forwarding generations, molecular markers aid in the speedy and exact identification of resistant plants. Choosing the best and most trustworthy markers will be quite useful in resistance breeding. In comparison to other solanaceous crops, information on eggplant genetics and genomics is limited. (Barik et al., 2020)

Due to the difficulty in chemical control of bacterial wilt and the presence of sexual barriers between eggplant and its wild relatives, cultivars grafted onto resistant rootstock is one of the most practical systems for eggplant cultivation. (Collonnier et al., 2001a) Because of increased pathogen densities due to intensification of production aspects, presence of susceptible cultivars to meet consumer demand, global spread of pathogens, and adoption of technologies for farmers with limited resources, the popularity of eggplant grafting has increased worldwide, particularly in countries such as Japan (65% of total area), Turkey (10% of total area), Korea, and Israel. (Yassin and Hussen, 2015) Rootstock breeding is a new area of study in which resistant sources or breeding rootstocks can resist diseases across strains. This method is potential for fighting bacterial wilt without changing genetic backgrounds. Because of its highly vigorous character, total graft compatibility with eggplant scions, (Moncada et al., 2013) and resistance to a wide range of soil diseases, particularly *R. solanacearum* (Rahman et al., 2002, Bletsos et al., 2003, Gousset et al., 2005) due to the presence of mechanical barriers generated in roots, (Clain et al., 2004) *S. torvum* is of significant importance as a rootstock for eggplant grafting.

Conclusion and Future Perspectives

This study unequivocally demonstrates the global significance of these diseases. Avoiding crop losses due to diseases helps greatly to enhanced

agricultural yield globally. Bacterial wilt disease caused by *R. solanacearum* is regarded as one of the most damaging diseases, producing significant output losses in eggplant. Grafting with resistant rootstocks eliminates the issue of changing genetic backgrounds by allowing scions to be grafted onto the rootstocks. Although *S. torvum* is the most recommended grafting rootstock due to its high compatibility, it has a long germination period and a low germination percentage. However, because poor and marginal farmers cannot buy grafted seedlings, breeding and producing varieties or hybrids is required.

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Functional Protein Domains Significant for MDM2-P53 Interaction

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Abstract

Ubiquitin E3 ligases are a well-known gene family that actively regulates the triple enzyme signaling pathway with the other two enzymes E1 (ubiquitin-activating enzyme) and E2 (ubiquitin-conjugating enzyme). The important role of E3 ubiquitin ligase is to catalyze the proteasomal degradation by transferring the ubiquitin-protein to bind to the lysine residues of targeted substrates. Deregulation in the ubiquitination pathway can lead to a variety of human disorders, particularly cancer. Because of their effective regulation and substrate selectivity during

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the ubiquitination cascade, E3 ligases are essential for influencing cellular homeostasis. E3 ubiquitin ligase could be a promising target in cancer therapy. The primary aim is to unravel the target binding sites of the RING-type murine double minute 2 (MDM2), which is known to be one of the vital E3 ligases involved in cancer development. Previous reports highlight that the MDM2 protein is over-expressed in several human malignancies. Since MDM2 is a negative regulator of the P53 tumor suppressor, thus the molecular basis of the interaction between MDM2 and P53 was investigated. A detailed analysis of the functional domains of MDM2 by bioinformatic tools and protein-protein docking studies revealed that the initial 150 amino acid residues of the N-terminal region of MDM2 are critical for interaction with P53 whereas the removal of the C-terminal region did not affect the interaction between the two proteins. Also, the deletion mutants of MDM2 where the zinc finger domains (241-480) were systematically removed did not alter the MDM2-P53 interaction.

Keywords: Ubiquitination, E3 ubiquitin ligase, ubiquitin-proteasomal degradation, MDM2

INTRODUCTION

The MDM2-P53 link has been a hot issue in the study and development of MDM2 inhibitors for cancer treatment. The tumor suppressor protein P53 is a potent anti-proliferative and pro-apoptotic protein that can damage healthy cells (Fang et al., 2000). The number of cellular P53 in non-stressed cells must be kept under control. MDM2 has long been recognized as having a crucial function to play in this control. MDM2 and P53 work together to form a complicated autoregulatory mechanism. In a feedback loop, the two proteins regulate each other's cellular levels (Yogosawa et al., 2003). P53 controls mdm2 gene production by interacting with its promoter (P1 promoter) (Uchida et al., 2005).MDM2 binds to and inactivates P53 when its levels grow

by directly blocking the P53 trans-activational region and directing the P53 protein towards ubiquitin-dependent proteasomal degradation (Pan et al., 2003). MDM2 and P53 were discovered to be linked via their N-terminal domains (Lee et al., 2010; Lai et al., 2001). The MDM2-P53 connection is diminished when the P53 C terminus is removed, altered, or acetylated (Poyurovsky et al., 2010). They demonstrated that a peptide fragment from the P53 C terminus directly binds the MDM2 N terminus in vitro utilizing a variety of approaches, providing a new interface for MDM2-P53 interaction (Ferreon et al., 2009). Because MDM2's binding site largely overlaps with P53's trans-activational domain, it significantly suppresses P53 transcriptional activity (Brady et al., 2010). The mdm2 gene is embryonic lethal in mice., however, mdm2/ animals may be successfully rescued by concurrently deleting the TP53 gene, which encodes P53 (Chi et al., 2005; Poyurovsky et al., 2010). Ubiquitin is added to proteins as a precursor tag for protein breakdown. Proteolysis occurs primarily through two main pathways firstly autophagy and secondly ubiquitin-proteasome system "UPS" (Brady et al., 2010). Both of which are required for the maintenance of the cell. Autophagy is an important adaptive mechanism in response to various cellular stresses through lysosome-mediated degradation of abnormal or excessive cellular proteins (Chi et al., 2005; Panigrahi and Satapathy 2020 a,b,c; Panigrahi and Satapathy 2021; Panigrahi et al., 2021; Sahoo and Satapathy 2021). UPS is a flux reaction and an important way to degrade short-lived, folded, and damaged proteins (Poyurovsky et al., 2010). As shown, UPS can regulate the breakdown of more than 80% of intracellular proteins and its dysregulation has been revealed in most cancer markers (Brady et al., 2010). Importantly, E3 couplings are an important part of the UPS and can regulate the enzyme chain's final step, including ubiquitin-activating enzymes (E1) and ubiquitin-conjugating enzymes (E2) (Chi et al., 2005). The main role of proteosomes is to degrade rapidly those proteins that are either defective or scheduled to be killed. So, they have to choose them properly by the cell markers known

as ubiquitin (Ub) (Brady et al., 2010). The oncogene CDC4 promotes a faulty surrounding response, which is required for the regulation of cyclic-independent kinases (Ferreon et al., 2009; Montes de Oca Luna et al., 1995). Therefore, the ubiquitin-proteasome degradation process is an important mechanism for controlling protein expression levels. Ubiquitination has also great effects on cell localization, protein interaction, and protein stability (Wang et al., 2011).

METHODOLOGY

Determination of protein sequences and putative protein partners

By using UniProt, the function, taxonomy, subcellular localization, disease/phenotypes, PTM/processing, expression, interaction, structure, family, domains, sequence, and isoforms of MDM2-Human E3 ubiquitin ligase (Q00987) and P53 were determined.

Determination of three-dimensional configurations of proteins

Three dimensional configurations of proteins of MDM2 and P53 were obtained from Swiss-Prot. It is accessed in the web server https://swissmodel.expasy.org/.

Visualization of three dimensional protein structures

The three dimensional structures of native proteins and interaction of proteins were primarily visualized by PyMOL. It is one of the few mostly open source model visualization tools available for use in structural biology. It uses openGL Extension wrangler library (GLEW) and free GLUT and can solve poission Boltzmann equation using adaptive poisson Boltzmann solver. Anyone can either compile an executable from the python licensed source code or pay for a subscription to support service to obtain access to precompile executable.

Protein-protein interactions

We used for protein–protein docking by using the server https://hdock.phys.hut.edu.in/.

RESULTS AND DISCUSSION

Domain organization and putative protein interactors of MDM2

MDM2 contains 491 amino acid residues (Figure 1).

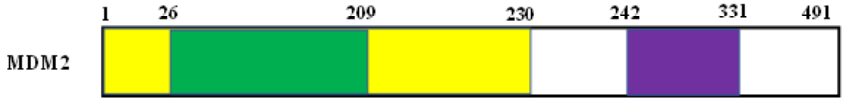

Figure 1: Functional domains of MDM2: 26-209= RING finger domain., 1-230= region 1: sufficient for binding with P53 and inhibiting its G1 arrest and apoptosis functions., 242-331= region 2: contains central acidic region required for interaction with ribosomal protein L5 and a putative C4-type zinc finger.

Putative protein targets of MDM2 are TP53, TP73, CDKN2A, UBE2D1, DAXX, EP300, USP7, MDM4, RPL5, and RPL11. The 3-dimensional configuration of target proteins: MDM2 and P53 were retrieved from PDB and Swiss-ProtKB (Figure 2-4).

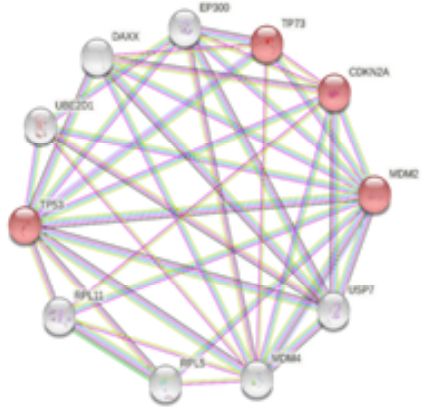

Figure 2: Putative protein interactors of MDM2

The protein-protein interactions of wild type MDM2, mutants with P53

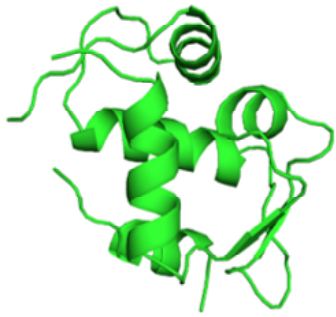

Figure 3: 3-d structure of MDM2

Figure 4: 3-dstructure of P53

The 3-d structure of MDM2 and P53 reveals that the WT MDM2 and its mutants largely are composed of á-helices and â-sheets, whereas the WT P53 primarily displays á-helices. Understanding the protein conformation is important to unravel the interaction of proteins. The interaction between wild type MDM2-P53 and the interaction between mutant types MDM2- P53 was obtained from H-DOCK. Using the h-dock module, the docking scores for protein-protein interactions were predicted. The wild type and domain mutants of MDM2 were included in this study (Table 1, Figure 5). It was observed that the docking score for the wild type MDM2-P53 was -245.75. The docking score for MDM2 (Ä1-120)-P53 was -204.64. the docking score for MDM2 (Ä121-240)-P53 was -280.67. The docking score for MDM2 (Ä241-360)-P53 was -245.36. the docking score for MDM2 (Ä361-480)-P53 was -247.98.From the dockings obtained from the h-dock, it is confirmed that the first 120 amino acid sequences of MDM2 play an important role in the interaction between MDM2 and P53. If the first 120 sequences are removed or blocked, then the docking score decreases,

and the stability between these two proteins becomes weak. The amino acids between 121 and 240 also have an important role in the MDM2-P53 pathway. If this region is mutated or deleted, then the docking score suddenly increases. From this, it is clear that the 121-240 regions have the regulatory property and function as a regulator.

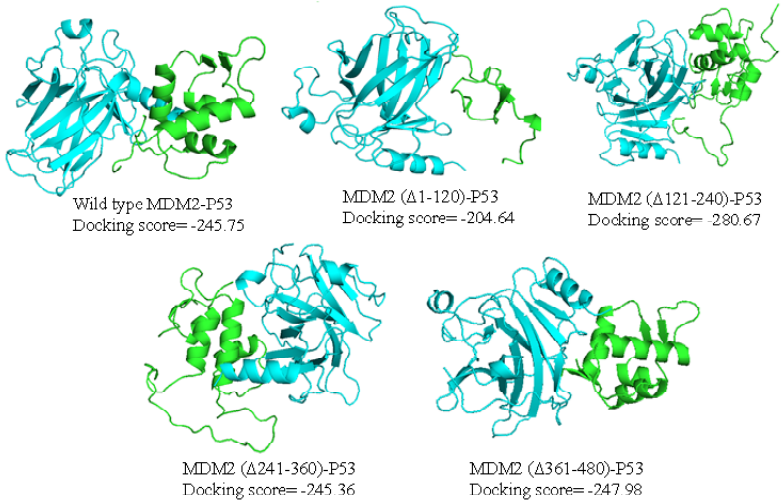

Figure 5: Interaction of wild-type MDM2 with P53

Table 1: Docking score of protein-protein interactions of wild-type and mutants of MDM2 with P53

Sl. No.	Interaction between MDM2 and P53	Docking scores
1	Wild type MDM2-P53	-245.75
2	MDM2 (Δ1-120)-P53	-204.64
3	MDM2 (Δ121-240)-P53	-280.67
4	MDM2 (Δ241-360)-P53	-245.36
5	MDM2 (Δ361-480)-P53	-247.98

CONCLUSION

Protein docking studies provides primafacie evidences with respect to the nature of interaction between proteins.The significance of the results directs way forward develop new strategies towards developing ligand(s) either to promote or restrict the protein interactions. The primary aim of the current study is to unravel the target binding sites of the RING-type murine double minute 2 (MDM2), which is known to be one of the vital E3 ligases involved in cancer development, so there could be a novel therapeutic inhibitor that could be designed to prevent the interaction between MDM2-P53. A detailed analysis of the functional domains of MDM2 by bioinformatic tools and protein-protein docking studies revealed that the initial 120 amino acid residues of the N-terminal region of MDM2 are critical for interaction with P53 whereas the removal of the C-terminal region did not affect the interaction between the two proteins. Also, the deletion mutants of MDM2 where the zinc finger domains (299-328 and 438-479) were systematically removed did not alter the MDM2-P53 interaction.There is also an important region which is worked as a regulator is 121-240 amino acid residues. This region should be activated during cancer treatment for the efficient restriction of interaction between MDM2 and P53. Our preliminary results paves way forward in improving our understanding on the functional aspects of critical protein factors such as MDM2 and P53. These evidences widens up the possibility of administering potential peptides when the P53 expression is inhibited.

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Interaction of Fat-1 With Tnf-A Via the N-Terminal Domain

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Abstract

Fat family members (FAT1, FAT2, FAT3, and FAT4) are human homologs of *Drosophila* Fat and are implicated in tumour suppression and planar cell polarity. Cellular homeostasis is largely maintained at the cellular level via transcription regulation, which can vary in response to physiological alterations. One of the most commonly altered genes in human cancer is FAT atypical cadherin 1 (FAT1), which encodes a protocadherin. FAT1 is thought to play a vital role in the maintenance

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of organ and cellular homeostasis, as well as activating a number of signalling pathways via protein-protein interactions, such as the Wnt/catenin, Hippo, and MAPK/ERK signaling pathways. Unregulated FAT1 expression can cause cancer and have a negative impact on prognosis. In this study, we focused on the structural and functional aspects of various domains and motifs of FAT1. Global bioinformatic databases resulted in streamlining a list of putative protein associates of FAT1. Since FAT1-mediated structural and functional alterations, as well as variations in FAT1 expression, contribute to disturbances in cellular homeostasis and result in patho-physiological disorders including cancer, we essentially focused on cancer-related genes functionally related to the FAT1. FAT1 is a huge protein composed of 4588 amino acid residues. By mutational analysis and further protein-protein docking studies using multiple bioinformatic tools it was confirmed that the N-terminus 1-100 amino acid residues are critical for interaction -with cancer-related genes including *Tumor necrosis factor*. Interestingly, it was found that the small peptides corresponding to the N-terminus domain 1-100 of FAT1 effectively interact with tumor-suppressor genes. These evidences widens up the possibility of administering potential peptides when the FAT1 expression is inhibited. Our preliminary results will pave way forward in improving the prognosis and treatment of patients with cancer.

Key words: FAT genes, tumor, patho-physiology, homeostasis, mutation, oncogene

INTRODUCTION

FAT1, FAT2, FAT3, and FAT4 are the genes that make up the human FAT family (Schreiner et al., 2006; Sun and Irvine, 2011). It was found in Drosophila in the 1920s as a result of a deadly mutation .Ft and Ft2, two FAT cadherin members found in Drosophila, are thought to tumour suppressors. "The Ft gene results in a Epithelial overgrowth

phenotype in Drosophila larvae, with mutational modifications affecting the wings, eye antenna, legs, glands, and genital imaging disc"(Peng et al., 2021). Fat2 is necessary for the formation and stability of ectodermal tubular structures. Any Fat2 mutation causes aberrant development of renal tubular structures in humans, including the absence of the trachea, gastric glands, and salivary glands. The complete coding sequence of FAT1, FAT2 and partial coding sequence of FAT3 was published (Mariot et al., 2015; Perugorria et al., 2019; Schwartz et al., 2003). The Complete coding sequence of FAT3 and FAT4 was reported (Camargo et al., 2007). The most similar to FAT3 is FAT1. Big proteins with extracellular cadherin repeats, EGF-like domains, and Lamnin G-like domains are encoded by both human and Drosophila FAT family genes. Invertebrates of the FAT family have distinct configurations in which the amount of laminin motifs and EGF-like motifs leads to the unique function. FAT1 and FAT4 have extracellular domain cadherin repeats, whereas FAT2 and FAT3 contain 32 and 33 repeats, respectively. "The condition of constant internal, physical, and chemical circumstances that biological systems maintain is known as homeostasis"(Mariot et al., 2015). All organisms' metabolic processes can only take place in highly precise physical and chemical conditions (Panigrahi and Satapathy 2020 a,b,c; Panigrahi and Satapathy 2021; Panigrahi et al., 2021; Sahoo and Satapathy 2021). The circumstances vary depending on the organism, and the chemical reaction occurs within the cell or in the interstitial fluid that surrounds the cells. "The best-known homeostatic systems in humans and other animals are regulators that keep the extracellular fluid composition constant"(Peng et al., 2021). The alternative homeostatic system, on the other hand, encompasses many components of human physiological controls and other entities in the body. Hyper and hypo conditions, such as hypothermia, hypotension, and hypertension, are usually preceded when the amount of variables is larger or lower than what is necessary by the process of homeostasis.From the previous reviews found that there are three domains that is helical, cytoplasmic and

extracellular in FAT1 gene. The helical domain is very less contained than cytoplasmic and extracellular topology in case of FAT1 cascade. The role of different domains is different in fat1 protein. The helical domain of FAT1 describes the mutational studies discovered in HNSCC which associated with research of two researchers and from the cancer genomics. The therapeutic studies pave the way to the result of activation of HNSCC by Notch signaling pathway. This domain also acts as a cadherin molecule to binds with the protein of fatty acid binding protein, contains high chain longevity and high affinity. New data from in vitro and whole animal research suggests that fatty acid transmembrane transport is mediated by proteins. FABPs appear to have a role in the cell's extranuclear compartments by transporting their ligands into the cytosol, where they interact with particular proteins. The extracellular domain plays major role that to consider as cadherin gene superfamily. FAT1 modulates cell contacts and polarity through regulating actin cytoskeletal structure at cell peripheries. Protein docking studies provides primafacie evidences with respect to the nature of interaction between proteins. The significance of the results directs way forward develop new strategies towards developing ligand(s) either to promote or restrict the protein interactions. The current study emphasizes the importance of FAT1 and TNF-A in maintaining cellular homeostasis and thus aligns with one of the objectives on United Nations formulated Sustainable Development Goals (SDGs); SDG3 which ensures healthy lives and promotes well-being for all at all stages. In this study, we focused on the structural and functional aspects of various domains and motifs of FAT1, primarily involved in interaction with Tumor necrosis factor-A (TNF-A).

METHODOLOGY

Determination of protein sequences and putative protein partners

Freely accessible database of protein sequence and functional information is found from Uniprot. It contains a large amount of

information about the biological function of proteins derived from the research literature. It is also a database which comprises many other databases such as Uniprot knowledgebase (UniportKB), the Uniprot reference clusters(UniRef) and the Uniprot Archive(UniParc). The Uniprot consortium collaborated with the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics(SIB) and the Protein Information Resources(PIR). EBI developed large resources of bioinformatics databases and services. SIB is the founding centre of the swissport group and maintains the EXPASY (Expert Protein Analysis System) server a central resource for proteomics databases and tools. Likewise PIR is the oldest sequence which to be considered as analysis and sequences the structure of protein. Also classifies the protein sequences in this tool. It is the database finds out from uniport shows that how one protein interacts with more than one protein. It is the functional protein association networks which also opened in NCBI by the server https://string.db.org/. Protein sequences and putative protein partners of FAT1 and TNF-A were retrieved from UniportKB and STRING.

Determination of three dimensional configurations of proteins

Protein Data Bank (PDB) was used for retrieving the protein structures. Swiss model is the part of Swiss-ProtKB is used as astructural bioinfromatics web server dedicated for homology modeling of 3D protein structure. Homology modeling is currently the most accurate methods to generate reliable 3D protein models and is routinely used in many practical applications. Homology modeling method makes use of experimental protein templates to build models for evolutionary related targets. Three dimensional configurations of proteins of FAT1 and TNF-A were obtained fromSwiss-Prot. It is accessed in the web server https://swissmodel.expasy.org/.

Visualization of three dimensional protein structures

The three dimensional structures of native proteins and interaction of proteins were primarily visualized by PyMOL. PyMOL is an open source

but proprietary molecular visualization system created by Warren Lyford DeLano. The private software company by DeLano scientific LLC dedicated to creating useful tools that become universally accessible to scientific and educational communities. Currently it is commercialized by Schrodinger,inc.as original software license was a permissive license they were able to remove it, new versions are no longer released under the python license, but under a custom license and some of the source code is no longer to released. PyMOL can produce high quality 3D images of small molecules and biological macromolecules such as proteins. According to original author by 2009, almost a quarter of all published images of 3D protein structures in the scientific literature were made using PyMOL. It is one of the few mostly open source model visualization tools available for use in structural biology. It uses openGL Extension wrangler library (GLEW) and free GLUT and can solve poission Boltzmann equation using adaptive poisson Boltzmann solver. Anyone can either compile an executable from the python licensed source code or pay for a subscription to support service to obtain access to precompile executable.

Protein-protein interactions

Protein- protein and protein-DNA/RNA interactions play a fundamental role in a variety of biological processes. Determining the complex structures of these interactions is valuable in which molecular docking has played a important role. HDOCK is the novel web module of our hybrid docking algorithm of template based modelling and free docking in which cases with misleading templates can be rescued by the free docking protocol. We retrieved our datas of protein- protein interaction by using the server https://hdock.phys.hut.edu.in/.

RESULTS AND DISCUSSION

The protein domain organization, three dimensional configuration and putative protein interactors of FAT1

In order to revealthe important function domains of FAT1,UniProtKBdatabase was considered which primarily provided significant information related to the protein of interest. FAT1, which is associated in various signalling events comprises of 4588 amino acid residues (Figure 1A, 1B). The three dimensional structures of the FAT1 and TNF-A proteins were generated using Swiss model. The proteins contain á-helices and â-sheets configured structures (Figure 1C, 2B). The putative protein interactors of FAT1 were retrieved from the STRING module, which highlighted ten numbers of proteins (Figure 1D). Since, TNF-A is involved in cell-cycle regulation and plays an significant role in maintaining cellular homeostasis, we opted to find out the functional domains of FAT1 involved in interaction with TNF A.

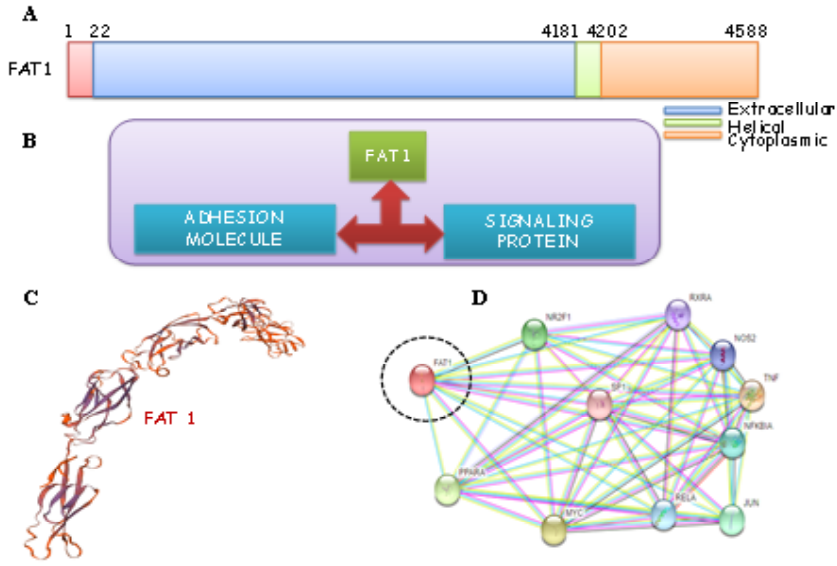

Figure 1: The domain organization of FAT1 (A); Major functional role of FAT1 in cellular physiology (B); 3-d structural configuration of FAT1 (C); Putative protein partners of FAT1 (D).

The protein-protein interaction of Wild-type FAT1, mutants with TNF-A

Using the H-DOCK module, the docking scores for protein-protein interactions were predicted. The Wild-type and domain mutants of FAT1 were included for this study (Figure 2). It was observed that the docking score for Wild-type FAT1-TNF-A was-285.10. The docking score for FAT1("1-100)-TNF-A was-253.92. The docking score for FAT1("101-200)-TNF-A was -265.32. The docking score for FAT1("201-300)-TNF-A was-264.72.The docking score for FAT1("301-400)-TNF-A was-287.29.The docking score for FAT1("401-500)-TNF-A was-263.42 (Table 1; Figures 3-8).The N-terminal 100 amino acids of FAT1 are vital for interaction with TNF-A since the deletion of initial amino acid residues resulted in weak interaction between FAT1("1-100)- and TNF-A with respect to wild-type FAT1-TNF-A interaction. However, it was observed that the docking score for FAT1("301-400)-TNF-A interaction was slightly elevated to -287.29 suggesting that the amino acid residues spreading from 301-400 residues did not affected the strength of interaction between two proteins rather it is quite relevant that this domain might regulate the interaction of FAT1 with TNF-A.

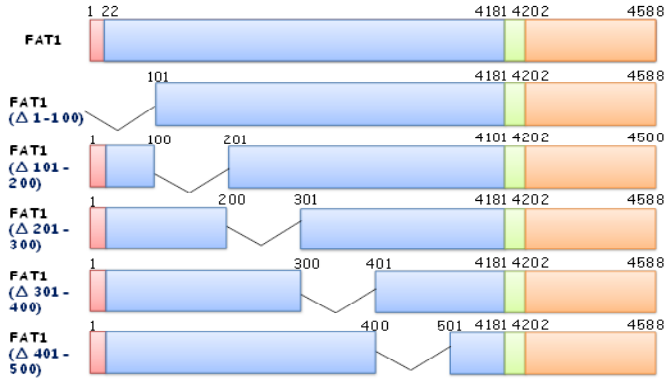

Figure 2: Wild-type and domain mutants of FAT1 used in this study for seeing their interaction with TNF-A.

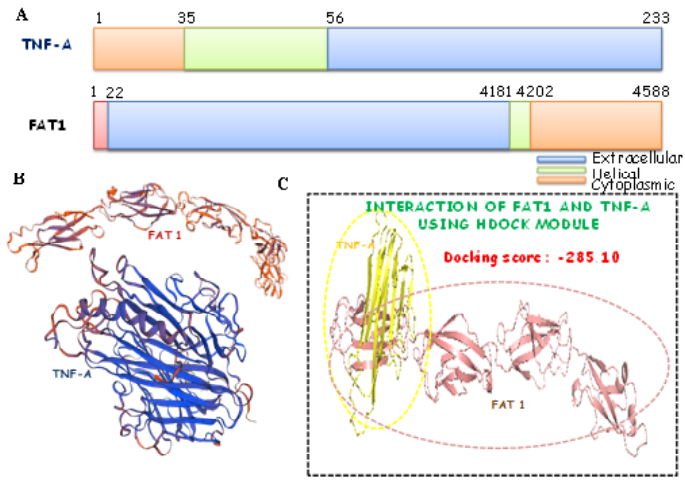

Figure 3: The domain organization of FAT1 and TNF-A (A); 3-d structural configuration of FAT1 and TNF-A (C); Interaction of FAT1-TNF-A (C).

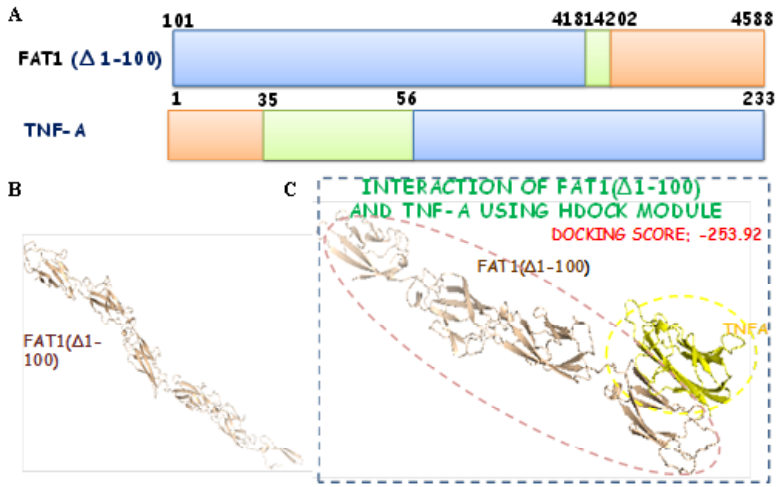

Figure 4: The domain organization of FAT1(^{3}%1-100) and TNF-A (A); 3-d structural configuration of FAT1(^{3}%1-100) (C); Interaction of FAT1(^{3}%1-100) -TNF-A (C).

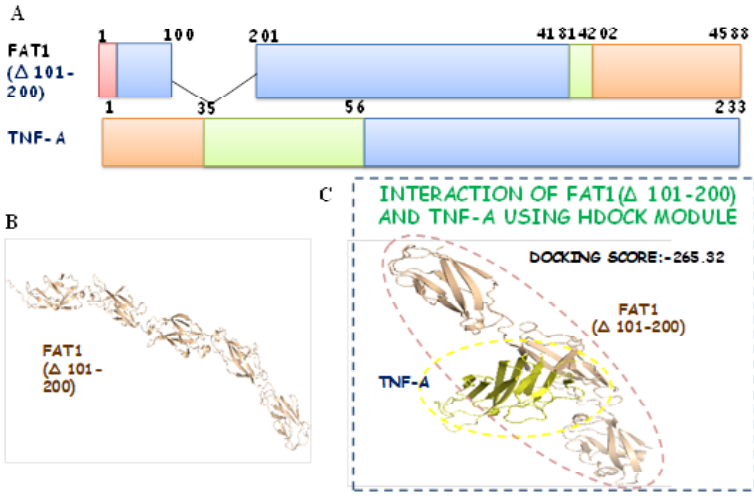

Figure 5: The domain organization of FAT1(^{3}%101-200) and TNF-A (A); 3-d structural configuration of FAT1(^{3}%101-200) (B); Interaction of FAT1(^{3}%101-200) -TNF-A (C).

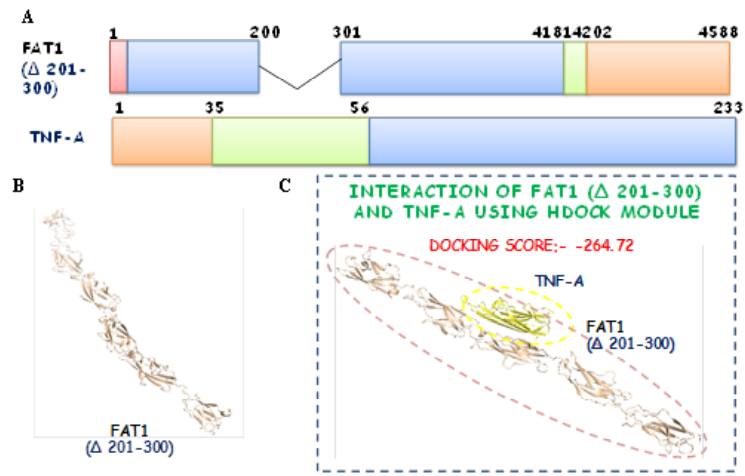

Figure 6: The domain organization of FAT1(^{3}%200-301) and TNF-A (A); 3-d structural configuration of FAT1(^{3}%200-301) (B); Interaction of FAT1(^{3}%200-301) -TNF-A (C).

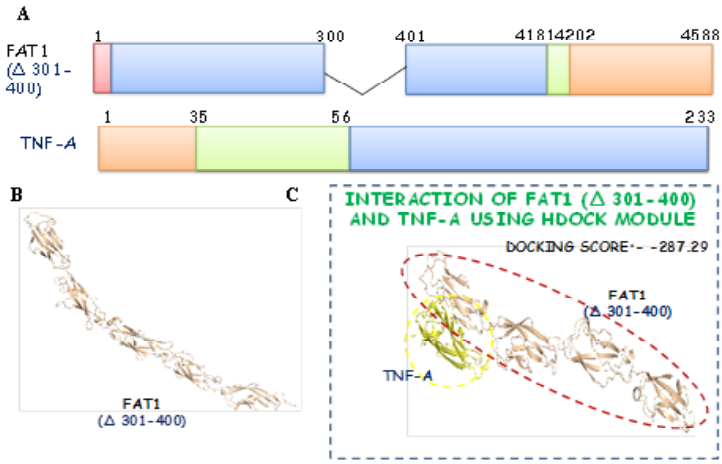

Figure 7: The domain organization of FAT1(^{3}%301-400) and TNF-A (A); 3-d structural configuration of FAT1(^{3}%301-400) (B); Interaction of FAT1(^{3}%301-400) -TNF-A (C).

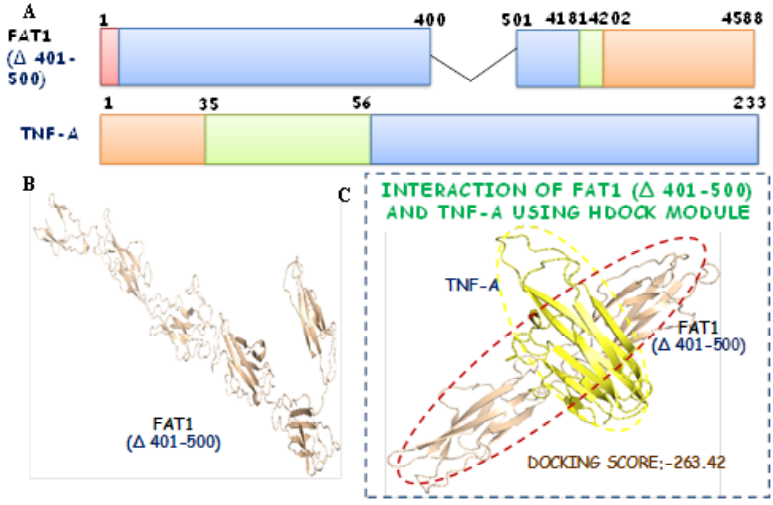

Figure 8: The domain organization of FAT1(^{3}%401-500) and TNF-A (A); 3-d structural configuration of FAT1(^{3}%401-500) (B); Interaction of FAT1(^{3}%401-500) -TNF-A (C).

Table 1: Docking score of protein-protein interactions of wild-type and mutants of FAT1 with TNF-A

SL. NO.	INTERACTION OF FAT1 AND TNF-A	DOCKING SCORE
1.	WT FAT1 and TNF-A	-285.10
2.	FAT1(Δ1-100) and TNF-A	-253.92
3.	FAT1(Δ101-200) and TNF-A	-265.32
4.	FAT1(Δ201-300) and TNF-A	-264.72
5.	FAT1(Δ301-400) and TNF-A	-287.29
6.	FAT1(Δ401-500) and TNF-A	-263.42

CONCLUSION AND FUTURE PROSPECTS

Using the H-DOCK module, the docking scores for protein-protein interactions were predicted. It was observed that the docking score for Wild-type FAT1-TNF-A was-285.10. The docking score for FAT1("1-100)-TNF-A was-253.92. The docking score for FAT1("101-200)-TNF-A was -265.32. The docking score for FAT1("201-300)-TNF-A was-264.72. The docking score for FAT1("301-400)-TNF-A was-287.29. The docking score for FAT1("401-500)-TNF-A was-263.42. The N-terminal 100 amino acids of FAT1 are vital for interaction with TNF-A since the deletion of initial amino acid residues resulted in weak interaction between FAT1("1-100) and TNF-A with respect to wild-type FAT1-TNF-A interaction. However, it was observed that the docking score for FAT1("301-400)-TNF-A interaction was slightly elevated to -287.29 suggesting that the amino acid residues spreading from 301-400 residues did not affected the strength of interaction

between two proteins rather it is quite relevant that this domain might regulate the interaction of FAT1 with TNF-A. Our results highlight the importance of various functional domains of FAT1 including the N-terminus 1-100 amino acid residues for interaction TNF-A. Interestingly, it was found that the small peptides corresponding to the N-terminus domain 1-100 of FAT1 effectively interact with tumor-suppressor genes. These evidences widens up the possibility of administering potential peptides when the FAT1 expression is inhibited. Our results steps way forward in improving our understanding on the functional aspects of critical protein factors such as FAT1 and TNF-A.

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Aberrant Transcripts and Cellular Defense Mechanisms

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Abstract

Nonsense-mediated mRNA decay (NMD) is an evolutionarily conserved surveillance mechanism in eukaryotes primarily deployed to ensure RNA quality control by eliminating aberrant transcripts and also involved in modulating the expression of a number of physiological transcripts. NMD, part of the mRNA surveillance pathway, is a major form of gene regulation in eukaryotes. The aberrant transcripts usually are suppressed under physiological conditions whereas, they start to express as soon as the NMD is compromised. The NMD process which is quite significant to ensure the cellular homeostasis is challenged under varied stress conditions which includes both biotic and abiotic stress. How a NMD challenged cell generates the stress response needs to

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be unravelled. The current review primarily focuses on NMD and the mechanism(s) involved in regulating the central dogma event, which essentially decides the fate of aberrant transcript towards their cellular decay.

Keywords: Nonsense-mediated mRNA decay, RNA, aberrant transcripts, central dogma.

Introduction

In multicellular organism a variety of NMD enhancer and suppressor proteins have been discovered all of which play a major role in NMD, but their functioning only for a set of specified target mRNAs (Gust et al., 2017; Ranf et al., 2017; Jones and Dangl, 2006; Boller and Felix, 2009; Jones et al., 2016). According to findings, NMD is considered as a branching route, in which corresponding branches control unique sets of transcripts in complex animals including vertebrates. Quality control is required at every level of gene expression, from transcription through posttranslational activities. Still, certain reiterations whose situations are changed under these experimental settings may not be direct targets of NMD, but rather contributory detriment through regulation of the expression of some natural NMD substrates. NMD can control its natural substrates via a number of styles. The exon junction complex (EJC) is deposited downstream of the physiological stop codon by splicing processes in the 3'UTR, and this is linked as a PTC. The actuality of a short reading frame in the 5'UTR, as well as differences in the splicing profile, might beget the NMD of the mRNA in question to be actuated. Eventually, NMD regulates certain mRNAs with a lengthy 3'UTR. This suggests that NMD must be regulated so as to permit the expression of genes that are ordinarily suppressed by NMD under particular physiological situations.

The NMD machinery

The core EJC complex provides an active region for additional components, for the apoptosis and splicing associated protein complex (ASAP). ASAP contains various factors like SAP18, RNPS1, and Acinus, along with CASC3 and UPF3B. NMD has been demonstrated to be

activated by RNPS1 (RNA-binding protein with serine-rich domain 1). In the nucleus, RNPS1 collaborates with the U1 and U2 snRNPs to recognise splicing sites. The N-terminal serine-rich region of RNPS1 is required for the activation of NMD, where RRM (RNA Recognition Motif) domain is present and a C-terminal section comprising of repeated sequences which are rich in serine and arginine (Dutta et al., 2017). RNPS1 increases mRNA degradation when linked with the 30-UTR of mRNA (Cesari et al., 2018; Nürnberger and Brunner, 2002; Boller and Felix, 2009; Bent and Mackey, 2007; Bigeard et al., 2015; Kunze et al., 2004; Hayafune et al., 2014) otherwise with the EJC for the period of splicing (Zhang and Zhou, 2010; Meng and Zhang, 2013; Pieterse et al., 2009; Cui et al., 2015; Peng et al., 2018; Yeh et al., 2015; De-La-Peña et al., 2012; Li et al., 2016; Huang et al., 2016). The SMG1 kinase is a member of the phosphatidylinositol-3-kinase-related kinase (PIKK) family It is having molecular weight of 410kDa (Mach et al., 2017; Ramirez-Prado et al., 2018; Nejat and Mantri, 2017; Ding and Wang, 2015; Jung et al., 2020; Couto and Zipfel, 2016; Raaymakers and Van den Ackerveken, 2016; Kourelis and van der Hoorn, 2018; Thomma et al., 2011; Boller and He, 2009; Stotz et al., 2014). SMG1 introduce a phosphate group to the serine/threonine-glutamine (SQ) motifs of UPF1 at free N and C terminal end region of CH helicase core (Liebrand et al., 2013; Albert et al., 2015; Doehlemann and Hemetsberger, 2013; Cook et al., 2015; Zipfel and Oldroyd, 2017; der and Joosten, 2019; Wieczorek and Obrêpalska-Stêplowska, 2015; Vaucheret et al., 2001; Agius et al., 2012). Also multiple phosphorylation sites have been discovered for UPF1 those are rich in leucine-serine-glutamine (LSQ) amino acid sequence (Gambino and Pantaleo, 2017). SMG1 kinase is closely linked to SMG8 and SMG9 in metazoans, generating the SMG1-8-9 complex (Unver and Budak, 2009; Pumplin and Voinnet, 2013; Rajeev Kumar et al., 2017; Holoch and Moazed, 2015; Du et al., 2015; Vergara and Gutierrez, 2017; McCue et al., 2015; Matzke and Mosher, 2014; He et al., 2009; Ma et al., 2015; Brodersen and Voinnet, 2006; Martínez-Pérez et al., 2017; Ruzicka et al., 2017).

NMD Mechanism

The UPF proteins like UPF1, UPF2, UPF3B make up the NMD event. The subunits of EJC like eIF4A3, MAGOH, RBM8A, and CASC3 (also

known as BTZ, MLN51 play an important role in slowed translation termination process which occur at PTC. These subunits help in the formation of ribosome-associated surveillance complex (Figure 1).There is presence of different factors like SMG1-8-9 kinase complex, UPF1, and eRF1 and eRF3 (SURF) in the conventional EJC-dependent model which is required for NMD (Zhang et al., 2010). The decay-inducing complex (DECID) is formed when ribosome-bound UPF1 interacts with EJC-bound UPF2 and UPF3B as part of the SURF complex (Zhang et al., 2010; Schuyler et al., 2016). The phosphorylation of UPF1 by the SMG1-8-9 kinase complex is influenced by DECID complex. UPF2 stimulates the ATPase and helicase activities of UPF1, which are required for NMD (Espinas et al., 2016; Akimoto et al., 2007; Dowen et al., 2012; Robert et al., 2011; Huang et al., 2016; Smale et al., 2014; Obata et al., 2015; Ramirez et al., 2018). The active phosphorylated UPF1 causes the NMD complexes to reorganise and also allowing for post-termination ribosome recycling (Shahbazian and Grunstein, 2007; Agius et al., 2012; Zhou et al., 2005; Zhou et al., 2010; Choi et al., 2012; Wu et al., 2008; Wang et al., 2017; Latrasse et al., 2017). SMG6 and SMG5-7 endonuclease are required forthe association of mRNA decay factors to the phosphorylation-sites (Wang et al., 2010; Singh et al., 2014; Defraia et al., 2013; Kim et al., 2008; Hou et al., 2015; Dutta et al., 2017; Alvarez and Avramova, 2005). The unprotected mRNA end is destroyed by the exonuclease of XRN1 and exosome complex (Alvarez et al., 2007). NMD is an mRNA monitoring cellular process that identifies and destroys transcripts with premature termination codon (PTC). PTC is a termination sequence that can be found anyplace at upstream region of the functional stop codon. The presence of PTC in the open reading frame (ORF) is most necessary for activation of NMD in mammalian cells. There is presence of two different ways NMD activation process in cell. The earliest event happens at the site of PTC which is one downstream splicing event at a space of 50-55 nucleotides (Berr et al., 2010) As the outcomes of intron splicing, the EJC is placed at 20-24 nucleotides upstream region of exon-exon junctions (Lee et al., 2016). Before recruiting UPF1, the EJC recruits the NMD core component UPF3X which sometimes termed as UPF3B and subsequently UPF2. The recruitment of UPF1 to EJC has been extensively researched,

while requiting of UPF3X and UPF2 is unknown. The collaboration of UPF1 with the CBP80 protein at the 5' cap, as well as the SMG1/ SMG8/SMG9 proteins with the eRF1/eRF3 release factors, have a great impact on recruitment of UPF1 during the former round of translation (Hu et al., 2014).

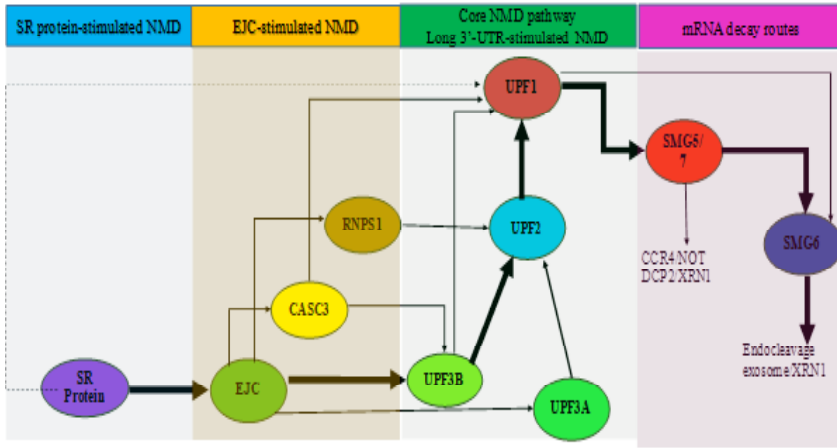

Figure 1: Possible NMD branches for decay of aberrant transcripts.

Cell regulation strategies

The basic NMD process is carried out by UPF proteins, exon junctions in 32 -UTRs which activate a significant branch of the vertebrate NMD system (Zou et al., 2014; Dhawan et al., 2009; Walley et al., 2008). EJC is a trimeric core complex made up of with EIF4A3, MAGOH, and Y14factors, which formed during splicing mechanism (March et al., 2008). 32 -UTR-bound EJC can trigger NMD system if the ribosome finishes translation at >50 nt upstream of the previous exon-exon junction (March et al., 2008). Despite the fact that the core EJC proteins are well-preserved among eukaryotes as are required for their survival,

the functional activities of NMD also has been proven in vertebrates (Berriri et al., 2016; Hilker et al., 2016; Lämke and Bäurle, 2017; Beckers et al., 2009)

UPF3B (or UPF3A) binds to a composite surface provided by the trimeric EJC core (Slaughter et al., 2012). According to current EJC-dependent NMD models The EJC downstream region of a terminated ribosome attracts UPF3B and UPF2, promoting the creation of the UPF complex and help in the process of UPF1 phosphorylation. Furthermore, EJCs can stimulate NMD ultimately by increasing the rate of translation occurred in both upstream and downstream region (Luna et al., 2012). During the cell differentiation process, a new gene is expressed and gets regulated by internal control mechanisms to remain activated throughout the process. The productivity of NMD has been explored in a variety of cell differentiation processes, including nervous system development. The Mutated NMD factors (UPF3X and UPF2) or EJC proteins induce obstruction in humans, according to preliminary findings (Rasmann et al., 2012). Regulation of NMD during the development of nervous system appears to be critical, as NMD has role to control axonal growth (Ramírez et al., 2017). The over-expression of the microRNAs miR-9, miR-124, miR-125, and miR-128 causes the appearance of UPF1, CASC3 and SMG1 to be suppressed throughout this process (Jaskiewicz et al., 2011; Mozgová et al., 2015; van Hulten et al., 2006). NMD is apparently suppressed in this way in order to allow the production of natural NMD substrates. SMG1 kinase is closely linked to SMG8 and SMG9 in metazoans, generating the SMG1-8-9 complex Dhawan et al., 2009. The kinase domain of SMG1, like all PIKKs, is made up of with a helical solenoid 'arch' present at N-terminal and a compact 'head' region at C-terminal. There are different types of domains found all found in the globular C-terminal 'head' region like FRAP domain, which has kinase activity, ATM domain having 1200 residues and a short FAT C-terminal (FATC) domain (Lee et al., 2016; Walley et al., 2008).

SMG1's insertion domain is distinctive from others, as it has the capacity to prevent kinase activity when combined with SMG8 and SMG9 (March et al., 2008). The N-terminal G-like domain of SMG8 is comparable to that the dynamin which is one type of GTPases. It is made up of with an eight stranded sheet in the centre, which is surrounded by seven helices, and a stem-like domain formed by a package of three helices (Berriri et al., 2016). The G-fold domain present in SMG8 and SMG9 are identical to each other, by using the identical part both the molecules form a stable pseudo-symmetric heterodimer structure. The ATP hydrolysis of SMG9 and UPF1 is thought to be driving this structural rearrangement of SMG1C, because SMG1C phosphorylation being the crucial steps in NMD. Langer and colleagues have enlightened the cryo-EM structure of SMG1C which are having the peptide rsidues just identical to SQ domain of UPF1. The SQ-domain peptide Q1079 of UPF1 is placed in a hydrophobic cage consisting of the activation region for SMG1 and the FATC domain which is a kinase active site for SMG1. A mutated glutamine residue suppresses phosphorylation process, which is the significance of Q1079. A residue of leucine which is hydrophobic in nature present at 1st position SQ-domain of UPF1 increases efficiency and selectivity of SMG1C's phosphorylation. In the lifecycle of mRNA, EJC has an important role having a constant core structure but a changing complement of peripheral proteins (Wang et al., 2010; Singh et al., 2014; Defraia et al., 2013; Kim et al., 2008). According to various studies it has been seen that EJC proteins such as RNPS1 and CASC3 affect the NMD paths like UPF2- and UPF3B-independent NMD pathways. The recent studies help to explain why these two proteins RNPS1 and CASC3have non-overlapping functions during NMD in case of mammalian cells (Li et al., 2016).

Future perspectives

Some mRNAs are targeted by NMD at early translational rounds whereas few during later phase, the underlying molecular mechanism

remains unclear. Few truncated proteins which are usually considered to be disadvantageous for the cell, are still synthesized before the aberrant mRNAs are decayed, what is the physiological role of those protein products. NMD which was largely considered simply a tool for RNA-quality control, now, is also related to be part of a complicated disease management strategy. Taken as a whole, a detailed molecular understanding of the NMD mechanism could lead to wide-ranging applications for improving cellular homeostasis and paving out strategies in combating pathological disorders.

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Messenger RNA Surveillance towards Cellular Homeostasis

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Abstract

Messenger RNA serves as quality control mechanism which differentiates the aberrant and non-aberrant transcripts. The synthesis of truncated proteins are restricted which would otherwise lead to cellular dysfunctions. The up-frameshift factors (UPFs) play a central role in executing the Nonsense-mediated mRNA decay (NMD) event, largely by recognizing and recruiting multiple protein factors resulting in decay of non-physiological mRNAs. NMD exhibits astounding variability in its competence across eukaryotes in both pathological and physiological and contexts. The detailed understanding on NMD and the underlying molecular mechanisms still remains blurred.

Keywords: Nonsense-mediated mRNA decay, cellular dysfunctions, up-frameshift factors.

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Introduction

Nonsense-mediated mRNA decay (NMD) is a conserved process in all eukaryotic cells, which acts as main surveillance mechanism. NMD quickly destroys mRNAs containing nonsense mutations to maintain the correct protein production. Fidelity mechanisms monitor genetic pathways, acting as proof readers to guarantee that data transmission is error-free. NMD also has a role in regulation, which is destroying a large percentage of transcripts produced by mutation-free genes (Figure 1). NMD controls around 10% of the transcriptome in vertebrates mainly in mammals (Mendell et al., 2004; Wittmann et al., 2006). Its mission is to track for premature termination codons (PTCs).The well-studied of all quality control systems is nonsense-mediated mRNA decay (NMD) (Hwang et al., 2021; Karousis et al., 2016; Nogueira et al., 2021; Popp et al., 2018). NMDs are detected during the first or pioneer translation cycle (Ishigaki et al., 2001). After the detection there is initiation of exonucleolytic and endonucleolytic pathways that delete mutant mRNAs (Eberle et al., 2009; Huntzinger et al., 2008; Lejeune et al., 2003). Another method for triggering NMD occurs during subsequent cycles of translation and is dependent on the distance between the polyA binding proteins C1 (PABPC1) and the first stop codon encountered by the ribosome (Buhler et al., 2006; Singh et al., 2008).

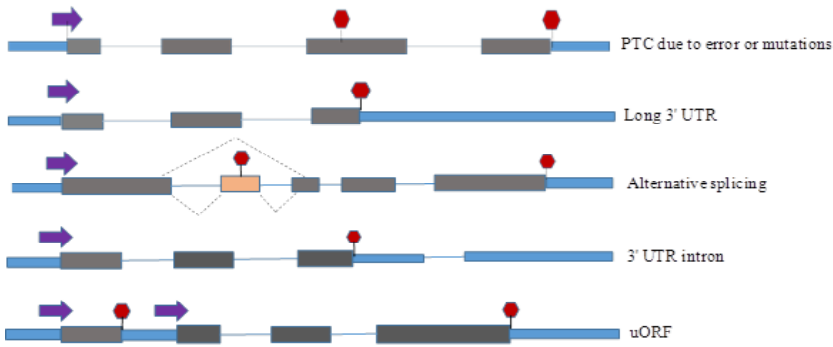

Figure 1: Gene features of NMD targets.

The up-frameshift factors

The most important NMD element is up-frameshift factor 1. UPF1 binds with m-RNA in a non-specific manner during coding sequence translation, but it is not happening during 30-UTR translation (Medghalchi et al., 2001;Weischenfeldt et al., 2008;Wittkopp et al., 2009; He et al., 1997; Pulak and Anderson, 1993; Conti and Izaurralde, 2005; Mühlemann et al., 2008; He and Jacobson, 2015; Gehring et al., 2005; Tarpey et al., 2007). UPF1 is a highly conserved eukaryotic protein of 125 kDa. UPF1 comprises of three core domains: a CH domain, which is rich in cysteine-histidine and a helicase domain with well-defined structures, also a SQ domain rich with serine and glutamine present at the free C-terminus end (Bao et al., 2016; Ge et al., 2016; Zhang and Krainer, 2004; Hogg et al., 2010; Zünd et al., 2013; Kurosaki et al., 2013; Hurt et al., 2013; Chakrabarti et al., 2011; Kadlec et al., 2006; Cheng et al., 2007; Clerici et al., 2009). The CH domain consists of two RING box like modules as RING1 and RING2 which are making a unique combination in compare to others (Gowravaram et al., 2018; Durand et al., 2016; Okada et al., 2012; Chakrabarti et al., 2014; Nicholson et al., 2014; Feng et al., 2017). Moreover, rather than impacting mRNA stability, human UPF1 has been shown an important role in the down-regulation of the MYOD, which is a transcription factor. It has a significant role during regulation of myogenesis, by increasing ubiquitination and subsequent degradation activities, which is enhanced by the proteasome (Joazeiro et al., 2019). However, it is unpredictable if UPF1 is act as the E3 ubiquitin ligase that may directly cause for MYOD ubiquitination (Powers et al., 2020). It's difficult to believe that E3 ubiquitin ligase activity of UPF1 is involved in the degradation of truncated polypeptides and nonsense mRNA-encoded polypeptides which are present in C terminal end. Blocked ribosomes allow the E3 ubiquitin ligase LTN1/ Listerin to poly-ubiquitinate the nascent polypeptide, which is then destroyed by the proteasome in different translational quality control

channels (Chu et al., 2021; Inglis et al., 2022; Chamieh et al., 2008; Weng et al., 1998). On the other hand, PTC-containing mRNAs are producing truncated polypeptides which fates are unknown (Figure 2). A reporter system with normal and PTC-carrying mRNAs that produce the identical polypeptide recently demonstrated that the nonsense-encoded protein is selectively destabilised. A regulatory system having normal and PTC-carrying mRNAs producing the same polypeptide recently demonstrated that the nonsense-encoded protein is selectively destabilised.

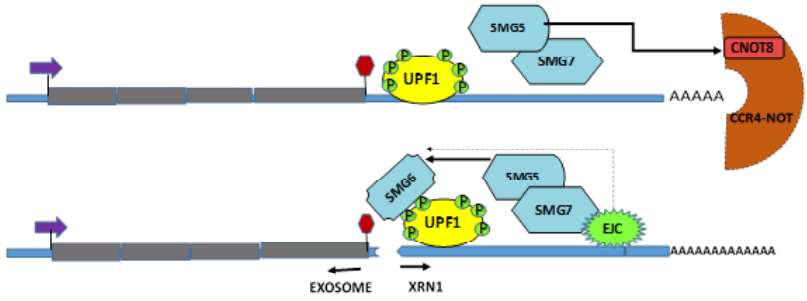

Figure 2: mRNA degradation after UPF1 activation.

Exon junction complexes

EJC core proteins and EJC-associated proteins are involved in mRNA metabolism, which includes proper splicing, export, mRNA localization, translation, and mRNA turnover, which includes NMD (Ge et al., 2016; Kishor et al., 2019, 2020; Fritz et al., 2022; Mabin et al., 2018; Gerbracht et al., 2020). The major RNA-binding component EJC is composed of eIF4A3 which is the DEAD-box RNA helicase. eIF4A3 has an important role in RNA-associated processes, which is a member of the helicase superfamily 2. It has two spherical domains which is made up with at least 12 extremely conserved series of motifs in each (Kadlec et al., 2004). The two domains of RecA perform a secure configuration in

the presence of ATP, which makes easy to binding with the sugar phosphate backbone of six consecutive mRNA nucleotides (Panigrahi et al., 2021; Melero et al., 2012; Sukarta et al., 2016; Buchwald et al., 2010). Due to a high hydrophobic attraction and well preserved interaction boundary both thee monomers MAGOH and RBM8A form a stable heterodimeric configuration (Kashima et al., 2006). Main function of the heterodimer is to lock eIF4A3 at exon-exon junctions of the mRNA which present in the 20-24 nucleotides upstream region (Bono et al., 2006; Andersen et al., 2006; Panigrahi et al., 2021; Shivaprasad et al., 2012; Singh et al., 2012). The ATPase and helicase activity of eIF4A3 is inhibited by the creation of EJC core compounds (Nguyen et al., 2012; Sato et al., 2008; Wallmeroth et al., 2022; Yi et al., 2022; Panigrahi et al., 2020). The folding of MAGOH contains a single domain made up of six anti parallel-strands having two long and one short -helices on one side (Zetoune et al., 2008). The N-terminal domain of RBM8A is conserved, which is required for MAGOH interaction. The RNA-binding like domain (RBD)is made up of with four-stranded antiparallel sheet having one helix on one side and two -helices on the other side (Kurihara et al., 2009; Viphakone et al., 2019; Linder et al., 2011; Andersen et al., 2006). Unlike other RBDs, RBM8A's RBD binds with MAGOH rather than RNA and also with the RBM8A's antiparallel-sheet present on the helical surface of MAGOH (Karasov et al., 2017). CASC3 (cancer susceptibility candidate gene 3) was previously thought to be the fourth EJC core factor as it acts together with eIF4A3 in a stable manner and also essential for EJC association during in vitro. The significance of CASC3 has recently been re-examined since the lack of CASC3 has no effect on EJC assembly in the nucleus during its early stages (Nielsen et al., 2009). CASC3 was recommended to functioning as an outer EJC protein which is essential for the destruction of EJC-dependent NMD substrates. The large no of alternatively spliced mRNAs aregoing under up-regulation in CASC3 CRISPR-Cas9 knockout cells, despite the fact is that the EJC configuration and EJC-dependent splicing both the events are independent of CASC3 (Le et al., 2000). CASC3 increases SMG6-dependent endonucleolytic cleavage occurat the terminal end codon in presence of SMG6. The SMG6 endonuclease possesses two conserved EJC-binding motifs (EBMs) and can act

together with the EJC molecule (Fribourg et al., 2003). It has been demonstrated that CASC3 has great efficiency to disturb the degradation process of NMD sensitive mRNA isoforms. UPF3B binds to the EJC in the nucleus region and transported together in the presence of EJC-bound mRNA, whereas UPF2 is drafted to EJC at the nuclear envelope by UPF3B afterward it has been exported (Ballut et al., 2005). According to new research CASC3 may have a role in boosting the interaction between UPF3B and EJC. This is consistent with findings link among CASC3 holding EJC and UPF3B (Sahoo and Satapathy, 2021). UPF3B association with the EJC was consistently decreased in CASC3 knockdown cells, while CASC3 over-expression increased EJC-UPF3B interaction in HeLa cells (Kashima et al., 2010). According to the configuration of different interaction with the EJC core are restricted to tiny areas of the two proteins. Also less-arranged areas of CASC3 and UPF3B may however, play a role in the EJC interaction. Wallmeroth and colleagues found that CASC3 act together with UPF2 in both wild-type and mutant cells by the using of two techniques which are co-immunoprecipitation (co-IP) and mass spectrometry (Garcia et al.,2014). Finally, CASC3 appears to be a significant centre for NMD factor.

Multiple Regulatory Pathways

As previously stated, the UPF3B-dependent branch plays an important function in nervous system of body (Lykke et al., 2001; Gehring et al., 2009; Sakashita et al., 2004). The UPF3B-dependent branch It is easily understood because of the monitoring feature and the methods by which activity may modified. Otherwise, those cells may depend more on a widespread UPF3B function (Imseng et al., 2018). UPF3B function can be restricted to certain cells/tissues by a variety of ways. To modify the NMD flow through this branch, one upfront strategy is to modulate the expression level of UPF3B. UPF3B mRNA levels are low in non-pluripotent cells as they are in pluripotent cells shows that UPF3B-dependent NMD level is decreased when cells develop through the pluripotent stage (Yamashita et al., 2001). A number of data support the function of canonical SR proteins in NMD since the initial information shows that over-expression of canonical SR proteins

increases the activity of reporter transcripts present in NMD (Zhang and Krainer, 2004). NMD may be enhanced by nucleo-cytoplasmic shuttling SR proteins (e.g., SRSF1) and nucleus-retained SRSF2 (Lykke et al., 2001). As a result, SR proteins are expected to induce NMD through a variety of ways. All conventional SR proteins, except SRSF2 act together with the RNPS1-EJC (Le et al., 2000). SR proteins and EJC may be able to increase the rate of NMD by making a bond with spliced RNAs. The concentration of SR protein binding sites around the EJC at non- canonical EJC binding locations, increase the EJC deposition by nucleus-retained SRSF2.Secondly, SRSF1 may interact with UPF1 at nucleus or cytoplasm region.As a result, SRSF1 can boost NMD without EJC, UPF2 and UPF3B components. SRSF1 may increase the activity of UPF1 by forming a bond with the phosphatase PP2A (Gehring et al., 2009). The over-expression of SRSF1 change the site of NMD in the pioneer round of translation (Imseng et al., 2018), implying one more mechanism for NMD control through enhanced translation. It's still unknown if NMD's UPF3B reliance is mediated by separate transcript that may influence translation process. In contrast to UPF3B, UPF2 is required for embryonic development in mice because deletion UPF2 results in death by embryonic day 9 (Weischenfeldt et al.,2008). The removal of UPF2 in knockout animals show inhibition of NMD and abnormalities during the development of all tissues, which indicates UPF2 plays an important role throughout development.The conditional deletion of UPF2 in mouse spermatocytes results over-expression of PTC-containing transcripts, but selective regulation of transcripts with lengthy 32 -UTRs (Garcia et al., 2014; Lykke-Andersen et al., 2001; Gehring et al., 2009; Sakashita et al., 2004). The discovery of partially mutated UPF2 in people with neurological problems suggests the dependence of UPF2 which varies by types of cell (Gehring et al., 2009). These genetic findings point to UPF2 having no function in some specific situations of cell. In HeLa cells, several NMD-targeted mRNAs are unaffected by knockout of UPF2, indicating that NMD is independent of UPF2or unaffected by lower amounts of UPF2 (Gehring et al., 2005). Furthermore, a mutated UPF1 protein lacks binding capacity with UPF2 but phosphorylated by SMG1 in these cells (Sakashita et al., 2004). NMD complexes are not reliant on UPF2, in UPF2-depleted HeLa cells (Yamashita et al., 2001).

Conclusion

How an aberrant mRNA is differentiated from a non-aberrant is critical and whether the aberrant transcript is targeted for decay or spared largely determines the effect of a disease-causing mutation. It signifies the importance of understanding the variability in NMD essentially for clinical interpretation of genetic variants and also to identify novel therapeutic approaches towards countering nonsense mutations-mediated genetic disorders. NMD selectively degrades a subset of transcripts harbouring PTCs and leaves the rest. NMD is linked with several cellular disorders including cancer. Multiple regulatory mechanisms involving a diverse array of protein factors including the core NMD factors, the UPFs contribute towards the activation and execution of NMD surveillance.

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