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Seed priming with sulphur oxidising bacteria for improving seed germination and vigour in sesame cv. VRI 4

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Abstract
Sulphur requirement for diverse soil types, oilseed crops and the role of microorganisms in making it available to plants are important to understand. The present study was conducted with the aim to evaluate the efficacy of seed priming with sulphur oxidising bacteria for improving seed quality attributes of sesame. The objective of the research is to find the suitable sulphur oxidising bacteria and optimise its concentrations for seed priming in sesame cv. VRI 4. The seed priming agents *viz*. Azophos (Azospirillum + Phosphobacteria) and two different Sulphur Oxidising Bacteria namely *Dyella thiooxydans* ATSB10 and *Achromobacter xylooxidans* ON9503241 were utilised for improving germination and vigour along with hydropriming and nonprimed seeds used as control. The seeds primed with sulphur oxidising bacteria shown 10 and 8 per cent increase in germination over non-primed seeds and hydroprimed seeds, respectively. Similar increase in other seed quality attributes *viz.,* speed of germination, germination, root and shoot length, dry matter production and vigour index were observed. The sulphur oxidising bacteria *Achromobacter xylooxidans* @ 6% for 8 hours of priming improved all the seed quality attributes in sesame VRI 4.

Keywords: Sesame, sulphur oxidising bacteria, seed priming, germination, vigour

Introduction
Sesame (*Sesamum indicum*) is an important oilseed crop which gain more popularity due to the healthy nutrient makeup of its oil. It is thought to have originated in Tropical Africa. Sesame is known as the "queen" of oilseeds because of its oil content (50–60%), protein (18–25%), carbohydrate and ash content (El Khier *et al.*, 2008) [9]. Because of its utility in food and medicine, it is widely cultivated all over the world.
Oilseed production requires sulphur which is responsible for its quality and oil content. Sulphur is involved in the synthesis of cysteine, methionine, chlorophyll, vitamins (B, biotin and thiamine), production of protein and oil, carbohydrate metabolism and it is also related to the growth and metabolism particularly due to its impact on proteolytic enzymes (Najar *et al.*, 2011) [18].
Most of the plants and microorganisms absorb sulphur in the form of sulphate, which goes through several changes before being incorporated into the primary components (Katyal *et al.*, 1997) [13]. The "sulphur cycle" describes how sulphide compounds in nature changes over time. The primary driving force behind all of these changes is the soil's microbial biomass, which serves both as a source and a sink for inorganic sulphates. They mineralize, immobilize, oxidize and reduce the elemental and other reduced forms of sulphur. Sulphur-oxidizing bacteria plays a key role in transformation of sulphur in soil (Joshi *et al.*, 2021).
The sulphur oxidising bacteria are a heterogeneous group of organisms with the capacity to oxidise inorganic sulphur compounds through the process of oxidation. This process produces sulphate that is accessible to plants, enhancing soil fertility, while the acidity created during oxidation aids in the solubilization of plant nutrients and enhances alkali soils (Hitsuda *et al.*, 2005) [12].
Seed priming is a technique that allows seeds to be partially hydrated without radicle emergence before re-drying to their original moisture content. Soon after sowing, primed seeds imbibe and restart metabolic processes, increasing the germination rate, germination uniformity, speed of emergence and vigour of the seedlings (Anwar *et al.*, 2012) [5].
A modern seed improvement method called biopriming involves soaking the seeds in a bacterial suspension for a pre-determined amount of time to allow the bacteria to penetrate into the seed (Abuamsha *et al.*, 2011) [2].

Bio-inoculant seed priming usually produces faster growth and more vigorous seedlings. It has been associated with elevated hydrolytic enzyme activity, reactive oxygen species (ROS), detoxifying enzyme activity, modifications in the levels of internal plant hormones and altered gene expression in plants all of which promote better plant development (Deshmukh *et al.*, 2020) [8]. Seed priming provides the best environment for bacterial colonization and inoculation in the seed (McQuilken *et al.*, 1998) [15].

The current study is intended to concentrate on the applicability of Sulphur Oxidising Bacteria (SOB) for seed priming in sesame seeds as it is a need of an hour for enhancing the seed quality and yield through enriched sulphur content.

Materials and methods

The seed materials of genetically pure fresh sesame cv. VRI 4 seeds required for the current study were procured from the Regional Research Station in Virudhachalam. The bacterial cultures *viz., Azospirillum, Phosphobacteria, Dyella thiooxydans* ATSB10 and *Achromobacter xylooxidans* ON9503241 were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. The laboratory study was conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

Five different concentrations *viz.*, 2, 4, 6, 8 and 10 per cent of *Dyella thiooxydans*, *Achromobacter xylooxidans* were prepared by dissolving 2, 4, 6, 8, and 10 ml of culture in 98, 96, 94, 92 and 90 ml of sterile distilled water, respectively. In case of Azophos, liquid formulation of *Azospirillum* and *Phosphobacteria* were mixed in equal proportions and five different concentrations of 2, 4, 6, 8 and 10 per cent were prepared similarly.

The seeds were first surface sterilized with 0.2 % sodium hypochlorite and then primed with Azophos, *Dyella thiooxydans*, and *Achromobacter xylooxidans* along with hydropriming. Under ambient condition, sesame seeds were soaked in an equal volume (1:1) of solutions for 8 hours. The seeds were collected from the solutions after 8 hours of priming and shade dried at room temperature to restore their original moisture content. The physiological parameters of non-primed and primed seeds were recorded. The experiment was carried out in a Completely Randomized Block Design with four replications (CRD).

The speed of germination was observed by recording the number of seeds that germinated each day. Following the procedure indicated in ISTA (2013), germination test was carried out using 4 x 100 seeds on roll towel medium in a room maintained at 25 ± 2°C and 95 ± 3 percent RH. The seedlings were evaluated at the end of the final count days i.e., after 6 days of seed test. The germination per cent was calculated based on the number of normal seedlings and the mean is expressed as a percentage.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

Ten normal seedlings from the germination experiment were chosen at random and used to measure the root and shoot lengths of the seedlings. Those selected seedlings were dried under shade for 24 hours and kept in an oven maintained at 85 ± 2^{0}C for 24 hours. The seedlings were weighed in a top pan balance after the drying period and the mean was expressed as g seedlings^{-10}. Vigour index (VI) was calculated using the method suggested by Abdul-Baki and Anderson (1973) [1] and expressed as a whole number.

Vigour index = Germination (%) x Total seedling length (cm)

Results and discussion

The speed of germination showed a significant difference among the different priming treatments. Seeds primed with *Achromobacter xylooxidans* @ 6 % for 8 hrs recorded higher speed of germination (15.5) followed by seeds primed with *Dyella thiooxydans* @ 8 % for 8 hrs (14.6) and Azophos @ 10 % for 8 hrs (13.8) whereas, the non-primed seeds observed a delayed germination (10.6) (Table 4). According to Bakonyi *et al.* (2013), plant growth-promoting bacteria have a favourable impact on germination and growth because they excrete phytohormones such as auxin, gibberellin, cytokinin and improve nutrient mobilization from the seed.

Similarly, significant difference in germination was observed (Table 4). The seeds primed with *Achromobacter xylooxidans* @ 6% for 8 hrs showed the highest germination of 94 per cent, which was 10% and 8% increase over nonprimed and hydroprimed seeds, respectively (Table 4). It was followed by seeds primed with *Dyella thiooxydans* @ 8 % for 8 hrs (92 per cent) which was on par with Azophos @ 10 % for 8 hrs (91 per cent) while nonprimed seeds had the lowest germination rate of 84 per cent followed by hydro priming for 8 hrs (86 per cent) (Table 1, 3 and 4). Grayston and Germida (1991) [11] observed that when the canola seeds were inoculated with sulphur oxidising bacteria the germination rate was increased by 24% over the control (un-inoculated seeds).

The difference in root length and shoot length were statistically significant in priming treatments. Seeds primed with *Achromobacter xylooxidans* @ 6% for 8 hrs measured the longest root length of 13.03 cm and shoot length of 5.81 cm followed by seeds primed with *Dyella thiooxydans* @ 8 % for 8 hrs with root and shoot length of 12.23 cm and 5.24 cm respectively. The nonprimed seeds had a shortest root and shoot length of 8.15 cm and 3.83 cm respectively (Table 4).

Oves *et al.*, found that *Achromobacter xylooxidans* considerably produced IAA (26 g mL^{-1}) when cultivated in a solution containing 100 g mL $^{-1}$ chromium. IAA (primary auxin) in plants control the processes that are involved in growth and development, including cell division, elongation and tissue differentiation (Fu *et al.*, 2015). Thus, IAA produced by *Achromobacter xylooxidans* is responsible for increased root length which results in increased seedling vigour. Similarly, *Pandoraeasputorum* ATSB28 (thiosulfate oxidizing bacteria) was examined by Anandham *et al.* (2008) [3] for characteristics relevant to promoting plant growth and found that *Pandoraeasputorum* increased the length of primary roots by 166 % in canola. According to research by Awad *et al.* (2011) [6] and Mohamed *et al.* (2014) [17], inoculating onion plants with sulphur oxidizing bacteria led to an increase in plant height, yield, and N uptake when compared to plants produced without inoculation.

Due to priming treatments, there was a statistically significant difference in dry matter production. The seeds primed with *Achromobacter xylooxidans* @ 6 % for 8 hrs produced higher dry matter (0.034 g seedlings^{-10}), which was comparable to *Dyella thiooxydans* @ 8 % for 8 hrs (0.032 g seedlings^{-10}), whereas nonprimed seeds produced the least dry matter (0.025 g seedlings^{-10}) (Table 4). Anandham *et al.*, (2007) [4] found that inoculating groundnut (*Arachis hypogaea* L.) cv. ALR-2 with both Rhizobium and *Thiobacillus* sp. (sulphur-

oxidizing bacteria) may result in plant growth promoting traits; co-inoculation with *Thiobacillus* sp. significantly boosted plant biomass, shoot length, and root length at 40 and 80 days after planting in pot and field experiments.
The vigour index was observed a highly significant difference among the priming treatments (Fig .2). Among the priming treatments, seeds primed with *Achromobacter xylooxidans* @ 6 % for 8 hrs recorded highest vigour index of 1761 and the lowest vigour index of 1010 was registered in nonprimed seeds (Table 4). Pourbabaee *et al.* (2020) found that *Thiobacillus* (sulphur oxidising bacteria) combined with elemental sulphur causes an increase in the oxidation of the elemental sulphur, which enhances the availability of nutrients in the soil which results in increased plant development and vigour.
Among the different priming treatments, seeds primed with *Achromobacter xylooxidans* @ 6 % for 8 hrs have registered better seed quality attributes *viz.*, speed of germination, germination percentage, root length, shoot length, dry matter and vigour index compared to other treatments.

Table 1: Optimising the concentration of Azophos for seed priming to improve physiological traits in sesame var. VRI 4

Treatments		Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
Control		10.7	84	8.15	3.83	0.025	1002
Hydro priming		12.9	85	10.45	4.36	0.027	1269
Azospirillum & *Phosphobacteria* (Azophos)	2%	12.9	86	10.43	4.41	0.028	1277
	4%	13.3	87	10.52	4.57	0.028	1308
	6%	13.5	87	10.87	4.66	0.029	1357
	8%	13.7	89	11.04	4.74	0.030	1399
	10%	13.8	91	11.93	5.13	0.031	1546
MEAN		12.96	87	10.49	4.53	0.028	1306
S.Ed		0.143	0.535	0.264	0.062	0.001	25.572
CD (P=0.05)		0.307	1.147	0.566	0.132	0.002	54.852

Table 2: Optimising the concentration of *Dyella thiooxydans* for seed priming to improve physiological traits in sesame var. VRI 4

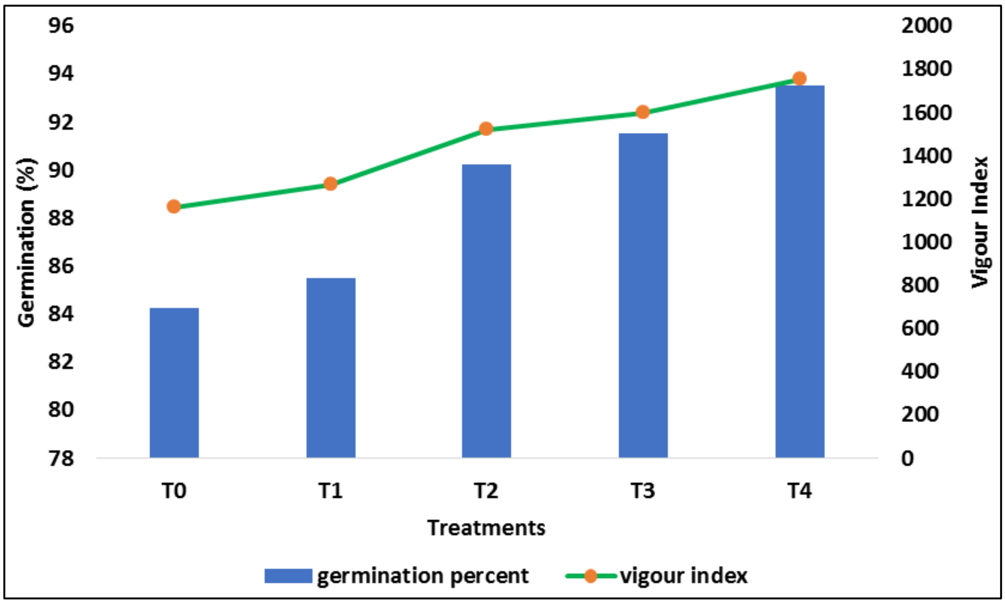
Treatments		Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
Nonprimed seed		10.7	84	8.15	3.83	0.025	1002
Hydropriming		12.9	85	10.45	4.36	0.027	1269
Dyella thiooxydans	2%	13.5	85	10.56	4.46	0.028	1282
	4%	13.9	88	10.85	4.62	0.029	1356
	6%	14.4	89	11.28	4.80	0.030	1422
	8%	14.6	92	12.35	5.31	0.032	1601
	10%	14.5	90	11.66	4.99	0.030	1504
Mean		13.51	87	10.76	4.63	0.029	1349
S.Ed		0.203	0.471	0.154	0.066	0.001	16.654
CD (P=0.05)		0.435	1.011	0.330	0.142	0.002	35.723

Table 3: Optimising the concentration of *Achromobacter xylooxidans* for seed priming to improve physiological traits in sesame var. VRI 4

Treatments		Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
Nonprimed seed		10.7	84	8.15	3.83	0.025	1002
Hydropriming		12.9	85	10.45	4.36	0.027	1269
Achromobacter xylooxidans	2%	14.0	88	10.67	4.51	0.028	1331
	4%	14.8	90	11.37	4.74	0.030	1445
	6%	15.4	93	12.86	5.58	0.033	1721
	8%	15.1	91	11.76	4.87	0.031	1508
	10%	14.9	89	10.47	4.57	0.030	1344
Mean		13.98	88	10.82	4.64	0.029	1372
SEd		0.270	0.563	0.094	0.055	0.001	13.146
CD (P=0.05)		0.579	1.209	0.202	0.119	0.002	28.199

Table 4: Influence of seed priming with different Sulphur Oxidising Bacteria for improving seed germination and vigour in sesame VRI 4

Treatments		Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g/10 seedlings)	Vigour index
Nonprimed seed		10.6	84	8.15	3.83	0.025	1010
Hydropriming		13.0	86	10.45	4.35	0.027	1269
Azophos	10%	13.8	91	11.72	5.15	0.031	1530
Dyella Thiooxydans	8%	14.6	92	12.23	5.24	0.032	1598
Achromobacter Xylooxidans	6%	15.5	94	13.03	5.81	0.034	1761
Mean		13.50	89	11.11	4.87	0.028	1430
SEd		0.135	0.592	0.112	0.051	0.001	13.712
CD (P=0.05)		0.288	1.261	0.239	0.108	0.002	29.227



T0 - Control T3 - *Dyella thiooxydans* 8 %
T1 - Hydro priming T4 – *Achromobacter xylooxidans* 6
T2 - Azophos 10 %

Fig 1: Optimization of concentration of Sulphur oxidising bacteria ongermination (%) and seedling vigour of sesame

Conclusion

The present study concluded that sesame seeds primed with various sulphur oxidizing bacteria can significantly improve seed quality parameters. The seeds primed with *Achromobacter xylooxidans* @ 6 % for 8 hrs recorded maximum speed of emergence, germination per cent, seedling length, dry matter production and vigour index followed by seeds primed with *Dyella thiooxydans* @ 8 % for 8 hrs compared with hydropriming and nonprimed seeds. This may be due to the favourable impact of plant growth promoting microbial inoculant as they excrete phytohormones, which results in improved seedling growth and vigour.

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