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RESEARCH ARTICLE

Genotypic response of silicon uptake ability in upland varieties of indica rice (*Oryza sativa* L.) and *in silico* analysis of silicon transporter gene (*Lsi2*) in var. Ghanteswari.

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Abstract

Rice is a crucial staple crop accumulating ~10% of (dry wt.) silicon. Si uptake abilities of seven upland indica rice genotypes (var. Annapurna, var. Badami, var. Ghanteswari, var. Lalat, var. Pathara , var. Sankar and var. Udaygiri) were screened with four Si concentrations (0.5, 1.0, 1.5 and 2.0 mM) at different day's interval (10d and 20d). Hydroponic culture revealed maximum relative growth of both shoot and root in 1.0 to 2.0mM of Si concentration. The indica var. Ghanteswari (140.9% at 1mM) and var. Lalat (139.1% at 2mM) showed maximum shoot growth, while maximum root growth was recorded in var. Ghanteswari (300% at 2mM) and var. Lalat (265% at 2mM). Molecular characterizations and allele mining of *Lsi2* gene was carried out in var. Ghanteswari using designed gene specific primer. Phylogenetic tree obtained from *Lsi2* gene of var. Ghanteswari suggested that this gene belongs to a separate domain and hence can act as a novel gene involved in Si uptake potential of indica rice. So, allele mining of gene can induce genetic manipulation of Si uptake capacity of the crop in general and rice in specific that might help in more Si transport and therefore improve their ability to overcome biotic and abiotic stresses.

Introduction:-

Silicon (Si) is considered to be the second most abundant element in earth crust (Bond and McAuliffe 2003) and accounts ~27.7 % of the total weight in soil after the 47% of oxygen (Datnoff and Snyder 2001). In the soil solution, silicon is mainly present in the form of uncharged monomeric silicic acid (H_4SiO_4) with the concentrations ranging from 0.1 to 0.6 mM (Epstein 1994) or up to ~ 0.8 mM at equilibrium (Lindsay 1979) at pH below 9 (Ma and Takahashi 2002). The significance of silicon to plants is highly variable, depending on the genotype and a whole raft of environmental conditions, so it was assigned a status of "quasi-essential" element, which confidently predicts rapid progress (Epstein and Bloom 2005). Rice shows the highest silicon accumulation up to the level of 10% of shoot dry weight which is higher than many macronutrients including nitrogen, potassium or phosphate as reported by Ma and Takahashi (2002) and Nhan et al. (2012) in the family Poaceae.

Silicon has shown to enhance growth and yield, promote upright stature, prevent lodging, promote favorable exposure of leaves to light and provide resistance to pest and diseases (both fungal and bacterial). Hence, the silicon accumulation in rice has many fold importance to promote good yield and high productivity. Thus, the interactions of silicon with rice has become of particular interest to several researchers (Savant et al. 1997, Mengel and Kirkby 2001, Ma and Takahashi 2002, Tamai and Ma 2003, Rodrigues et al., 2004). Under controlled conditions, application of silicon to rice plants increased resistance to blast against *Pyricularia grisea* and *Magnaporthe grisea* (Kawashima 1927). However, Datnoff et al. (1997) reported 3% to 5% of silicon was the minimum tissue levels

needed for disease control in rice. Supply of silicon in rhizosphere was not only been effective, but also "silicon supplementation in the form of external foliar treatments showed increased pathogen resistance of plant species (Richmond and Sussman 2003). Industrial by-products containing silicon were been used to benefit plants for increase rice disease resistance time to time (Jones and Handreck 1967, Savant et al. 1997, Pereira et al. 2004). Genotypic variation of silicon uptake and accumulation in the roots were reported by Parry and Kelso (1975) that revealed that silicon interacted with polyphenols in xylem cell walls and affected lignin deposition and biosynthesis. It is hypothesized that as water is transpires from the plant, silicic acid accumulates and forms colloidal silicic acid, then amorphous silica ($SiO_2.nH_2O$), which polymerizes at high concentrations (> 2mM), thus creating a rigid polymer within the plant (Jones and Handreck 1967, Gao et al. 2006). Most of the plants, particularly dicotyledons are unable to accumulate high levels of silicon in their shoots. Uptake of silicon appears to be passive in the Family Gramineae (Jones and Handreck 1967), but in hydroponically grown rice plants, Ma and Yamaji (2006) and Van Soest (2006) found active uptake of silicon. So, studies indicated that silicon is transported passively in the transpiration stream and is deposited at sites of high transpiration (Wiese et al. 2007). Silicon is concentrated in epidermal tissue as a fine layer of silicon–cellulose membrane and found to be associated with pectin and calcium ions. As silicon concentration in plant sap increases, monosilicic acid is polymerized in form of silicon gel. Silicon deposits 0.1μm thick between the cuticle and endodermal cells in rice (Ma and Takahashi 2002) as well as in guard cells around stomata in blueberry (Morikawa and Saigusa 2004). Silicon found reduce the transpiration rate by 30% in rice, which has a thin cuticle (Ma 2004). Lux et al. (2002) found high root endodermal silicification in sorghum cultivar that leads to drought tolerance.

Two silicon transporters for silicic acid, i.e. *Lsi1* and *Lsi2* were reported in japonica rice varieties (Ma et al. 2007). *Lsi1* is an influx transporter responsible for Si transport from the external solution to the root cells which is belongs to a Nod26-LIKE MAJOR INTRINSIC PROTEIN2 (NIP2) subgroup in the NIP subfamily of aquaporins and expressed in roots, more intensively in the mature regions than in the root tips (Ma and Yamaji 2007). On the other hand, *Lsi2* is an efflux transporter involved in Si transport from the root cells to the apoplast (Ma et al. 2007), is a putative anion transporter and was also constitutively expressed in the roots. These two transporters play a crucial role in silicon uptake because disruption of either of them resulted in a significant decrease of silicon uptake in japonica rice varieties. Comparative analysis of the sequences and gene expression of *Lsi1* and *Lsi2* Si transporters of two japonica varieties 'Nipponbare' and 'Kasalath' showed that the open reading frame of these two genes was the same in both varieties. So, there expression levels may be controlled by a number of factors such as promoter sequence and transcription factor that are yet to be identified. However, the uptake abilities of silicon in different indica varieties have not yet been reported so far. A comparative analysis of silicon is very essential to look in to the genotypic response of silicon accumulation of different indica rice to identify the maximum accumulator, which will provide maximum biotic and abiotic resistance in rice varieties. In this study, seven indica rice varieties were tested for silicon upake ability in hydroponics and characterization of *Lsi* 2 gene was done in highest silicon accumulator var. Ghanteswari – a promising high yielder upland indica rice.

Material and methods:-

Plant materials and growth environment:

Seven upland rice varieties namely var. Annapurna, var. Badami, var. Ghanteswari, var. Lalat, var. Pathara and var. Sankar var. Udaygiri developed in Orisssa University of Agricuture and Technology, Bhubaneswar were used for silicon uptake experiments. About 150 seeds of each variety were disinfected in 0.5% (w/v) fungicide Bavistin for 30 min with continuous stirring, then rinsed and soaked in deionized water at room temperature for 1d. After 5d when the seeds show uniform-germination, 20 seedlings of each were transferred to 5 plastic cups containing 20 ml of Yoshida's nutrient solution (Yoshida 1975, pH 5.0) which was renewed every 4 d interval.

Silicon uptake study:

Each 20-seedling in Yoshida solution was raised by fortifying with four different concentrations of silicon 0.5, 1.0, 1.5 and 2.0 mM Si along with control (0 mM). *Diatomaceous earth* (opal, $SiO_2 \cdot nH_2O$) was used as the source of silicon. The plants grew in controlled-environment growth chamber. After 10d and 20d, 3 seedlings from each variety were selected and the roots- shoot growths were measured and recorded for growth analysis. After 30d of treatment the samples were dried further to estimate the silicon content.

Root-shoot growth analysis:
Growth parameters of all seven varieties in different concentration were recorded and analyzed. The root-shoot growths were measured at 10d and 20d intervals and the relative shoot-root growth was calculated using following **formula:-** Relative growth (%) = Treatment growth/ Control growth × 100.

Determination of silicon content:
After 30d, hydroponically grown samples of different varieties at different concentrations were dried in oven at 70˚C for two days and used in further estimation. Tissue analyses was done by oxidation of plant material to release the various forms of silicon through wet-oven induced digestion (Elliot and Snyder 1991) and an analytical technique to quantify the amount of silicon released by colorimetric molybdenum blue method (Hallmark et al. 1982). Briefly, silicon content of rice seedlings was estimated using UV/Vis spectrophotometer. The samples digested in water bath were first diluted and then prepared for the colorimetric test by adding ammonium molybdate, to estimate the monosilicic acid content. The prepared samples were kept for one hour, for colour development (blue) and absorbance was taken at 630nm.

Primer designing of silicon transporter gene (*Lsi2*):
NCBI (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov) database was used for the primer designing. Nucleotide sequences of all possible silicon transporter genes (*Lsi1* and *Lsi2*) were searched and retrieved from databases of NCBI (http://www.ncbi.nlm.nih.gov/) through Entrez for *Oryza sativa* group. The conserved region obtained from the nucleotide sequences was used as input for designing primer. Primer3 (http:// frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi) software developed by Rozen and Skaletsky (2000) was used to design forward and backward primers having optimum parameters (%GC, Tm etc) and absence of secondary structures (Hairpin, dimmer, loop etc), i.e. SiF1-TCCATCGACCTCCCAATCCT and SiR1-ATGTGCTGCCAGAAGGTGA.

Orthologous gene cloning and sequencing:
These primers were synthesized by Bangalore Genei Pvt. Ltd., Bangalore, India for homology based gene cloning through PCR. PCR conditions were optimized in order to get a single sharp amplicon corresponding to silicon transporter gene (*Lsi2*). The amplified product was separated through electrophoresis and later eluted using PCR DNA fragments extraction Kit (Geneaid) following Vogelstein and Gillespie (1979). Finally, the samples obtained were sequenced with 96 capillary high throughput sequencer; ABI 3730 XL system following modified Sangers' dideoxy method at Xcelris Genomics Ltd., Ahmedabad 380054, India.

Blast:
All the sequences obtained where subjected to homology search in BLAST (Basic Local Alignment Search Tool). This algorithm compared the primary biological sequence information present either as the amino acid sequences of proteins or the nucleotides of DNA. BLAST search enables us to compare a query sequence with a library or database sequences, and identify or predict the nature or function of the query sequence.

Structural and phylogenetic analysis of silicon transporter gene:
All PCR amplicon sequences were subjected to Multiple Sequence Alignment (MSA) using ClustalW program at the EBI ClustalW server (http://www.ebi.ac.uk/ClustalW/) and muscle programme (www. ebi.ac.uk/Tools/msa/muscle/) by keeping parameters as default. MEGA (Molecular Evolutionary genetic analysis) 5.5 software was used for phylogenetic analysis [version 3.1; http://www.megasoftware.net] (Kumar et al. 2004). The phylogenetic tree was constructed using the Neighbhor joining method with bootstrap test. 3D Model validations of the predicted structures were done through Structural Analysis and Phyre Server.

Result and discussions:-

Silicon uptake and accumulation:
Comparison of silicon uptake ability was studied in seven indica varieties shows some interesting facts. A relative shoot-root growth analysis revealed that maximum relative growth of shoot and root was observed in Si concentration ranging from 1.0-2.0mM (Figs. 1a-1e). High shoot growth as well as root growth observed in var. Ghanteswari (140.9% at 1mM) and var. Lalat (139.1% at 2mM) out of the seven at 10 days of growth as compared to control without any Si treatment (Fig. 2a). Shoot growth was significantly very promising in var. Pathara at 1.0mM Si concentration at 10 days of growth. Whereas at 2.0mM Si inhibit shoot growth in var. Annapurna, var. Badami, var. Pathara, var. Shankar and var. Udaygiri as compared to respective non treated rice varieties at 10 days

of growth. Maximum relative root growth was shown in var. Ghanteswari (300% increase) followed by var. Lalat (265% increase) at 2mM as compared to control at 10 days of growth (Figs. 1d,1e and 2b). Rest of the varieties could not showed significant result with regard to root growth in different concentration of Si treatment (Fig. 2b). It was also observed that at a higher concentration of silicon, shoot growth inhibited; hence an optimum silicon concentration (1.0mM-1.5mM) found beneficial in each variety which enhance adequate plant growth. However, the genotypes like var. Annapurna, var. Badami and var. Shankar showed poor response towards Si-application and intake (Figs. 2a & 2b). However, var. Pathara showed high amount of shoot growth in 1.0mM Si concentration at 10 days of growth without significant root growth. Same trend of shoot and root growth were noted in 20 days old seedlings in var. Pathara (Figs. 3a & 3b). The trend of relative shoot growth was also found same in 20 days of growth as in found in 10 days of growth (Fig. 3a). As per the relative root growth is concerned, it was found that significant higher root growth ~one fold increase was noted in var. Ganteswari, var. Lalat and var. Udayagiri in 0.5mM to 1.5mM in 10 days of growth as compared to non treated plants. Hence, relative shoot and root growth was found significantly higher as compared to respective controls in case of var. Ghanteswari, var. Lalat in 1.0mM concentration of silicon.

Root: shoot ratio:

The highest root: shoot length ratio (threefold increase) was observed in 10 days old seedlings of var. Ghanteswari, var. Udaygiri and var. Lalat (two fold increase) at 2.0 mM Si treatment which is very significant as compared to control and other varieties too (Fig. 4a). Same trend was observed in the above three varieties at 20 day old seedlings at hydroponic (Fig. 4b). In all the concentration var. Shankar showed ~ 0.5 fold increase in root: shoot ratio whereas var. Annapurna, var. Badami, var. Pathara could not show very significant increase of irrespective of days of growth (Figs. 4a and 4b). Although 3 fold increase of root: shoot ration was recorded in var. Ghanteswari and var. Udaygiri, but the yield of rice per hector was significantly more in var. Ghanteswari as compared to other studied varieties of rice. At all the concentration of silicon var. Badami showed drastic root:soot ration as compared to untreated control that indicated the retardation of root growth as compared to shoot growth in this variety and high sensitiveness against silicon.

Silicon estimation:

Silicon content measurement found some significant result about silicon uptake. It was noticed that the varieties showing higher growth under Si-influence accumulate more silicon like var. Ghanteswari (six fold) in 1.0 mM concentration at 20 days of growth (Fig. 5). A moderate accumulation of ~ three fold as compared to untreated control in var. Lalat, var. Pathara followed by ~2.5 fold accumulation in var. Annapurna and var. Udaygiri at 1.0 mM concentration. Hence, the result suggested a positive correlation between the plant growth and silicon accumulation per gram dry wt. of plant. It also indicated a saturation level for Si-uptake which varies within genotypes ranging between 1.0-2.0mM concentrations. However, growth was stunted with less silicon content in var. Badami, var. Shankar, var. Udaygiri. 'Annapurna' showed high accumulation of Si at 1.0mM, which could not show significant growth that, might be due to genetically controlled silicon uptake mechanism in this variety. As var. Ghanteswari showed pronounced effect on root and shoot growth further molecular analysis of Si transporter gene (*Lsi2*) was carried out in this variety in detail. This variety has high yield potential as a midland rice variety having local importance with high grain yield of 35q/ha.

The specific mechanisms responsible for silicon ability to increase biotic and abiotic tolerance are not fully understood, though thickening of the silicon layer in the cuticle and improved stomata control have been suggested as contributing factor by many researchers. A series of suggestion were given to explain the possible mechanism of silicon induced resistance. Yoshida et al. (1962) and Ishiguro (2001) suggested that the enhanced host resistance of silicon-treated plants, to be the consequences of a greater resistance to pathogen penetration of host tissues due to the accumulation and polymerization of silicic acid [$Si(OH)_4$] in host cell walls which physically block penetration by plant pathogenic fungi. According to Volk et al. (1958), silicon might form complexes with organic compounds in the cell walls of epidermal cells, therefore increasing their resistance to degradation by enzymes released by *M. grisea.* In contrast, silicon might induce host resistance by triggering a cascade of defense mechanisms of plants, leading to the accumulation of antifungal compounds such as phytoalexins and pathogenesis-related proteins (Fawe 1998, Cai et al. 2009). Molecular and biochemical detections show that silicon can activate the expression of defense-related genes and may play important role in the transduction of plant stress signal such as salicylic acid, jasmonic acid and ethylene in plants other than rice. Further, the silicon-induced cell wall fortification results from a difference in the ability of the roots to take up silicon, which results from the difference in the expression level of Si

transporter genes (Deren 2001). This gave an evolutionary relationship between the japonica and indica rice ecotype for silicon uptake ability.

Molecular characterization of silicon transporter gene:

All these studies conducted on silicon accumulation and uptake indicated the presence of a "Silicon transporter gene" in rice plant system. Ma et al. (2004) suggested that at least two transporters are involved, one is located on the plasma membrane of root cortical cells (SIT1, silicon transporter 1), which transport silicon from external solution to the root cortical cells. The other is located on the plasma membrane of xylem parenchyma cells (SIT2, silicon transporter 2), which is responsible for releasing silicon into the xylem. Dai et al. (2005), identified QTLs controlling the silicon concentration in different organs of rice, but so far no QTLs controlling the silicon concentration of whole rice shoot have been reported. The QTLs identified provided the genetic basis for further research on silicon uptake ability in rice in various genetic backgrounds and to identify some novel major QTLs or genes. Information of such type of Si transporter genes is till now scanty in indica rice. So, these research aims to identify and isolate the transporter gene involved in silicon accumulation in indica genotypes.

The present investigation focuses on the molecular characterizations for 7 promising *indica* rice varieties. These 7 varieties were subjected to PCR amplification by designing the gene specific primer for *Lsi2* gene using the nucleotide sequences found in the databases. The Si transporter gene amplified consists of a sharp and bright fragment of 1200 bps, approximately (Fig. 6). All seven fragments were obtained by elution of 1200bps band. These gene sequences found from amplified fragments from gel and subsequent elution were subjected to homology search through BLAST of NCBI. A BLAST search detected high homology of 97% with the 'Citrate transporter family protein gene' of *Oryza sativa* cv. *indica* group. This gene also showed 97% homology to the sequence of 'silicon transporter gene (*Lsi1* and *Lsi2*)' reported in *Oryza sativa* cv. *japonica* present in the database. Then, the partial sequences obtain from the eluted bands were align with the homology sequence obtained through BLASTn in order to determine the conserve sequences. The MSA (ClustalW) of protein sequences of query and homology sequence obtained from BLASTx showed several conserved domains (Fig. 7). These conserve domains are located on α-helix structure. Few gapping were found during the protein alignment, which could a reason for the variation in proteins produced by the gene. The phylogenetic analysis of all align sequence deduced the homology in the consensus sequences of the *Oryza japonica* & *Oryza indica* obtained from database and the seven genotypes under investigation, i.e. var. Annapurna, var. Badami, var. Ghanteswari, var. Lalat, var. Pathara and var. Sankar var. Udaygiri. The varieties or genotypes showing high silicon uptake and accumulation were group or clustered together and the poor uptakers were separated. But, var. Ghanteswari showing highest uptake and accumulation was placed differently in the tree representing a mutation or novel change in gene sequence. Hence, it could be considered as a novel gene, identified in the indica genotypes of the rice which is constantly responsible for better silicon uptake potential of plant (Figs. 10 and 11). The gene sequence was again clustered with the Si transporter genes reported in other crops to understand and mark its evolution.

The open reading frame (ORF) of the gene under investigation (var. Ghanteswari) consists of 902 bps coding for 298 amino acids. The amino acid sequence of *Lsi2* gene showed 87% similarity and 79% coverage with reported citrate transporter family protein of *Oryza indica* group and 86% similarty and 93% query coverage with the putative ion transporter in *Oryza japonica* group. The 3D structures of silicon transporter gene of var. Ghanteswari depicted the presence of 10 entangled α –helix, all are connected and packed together in different orientation (Figs. 8a and 8b). The protein basically consists of 84% α-helix, without any β-strand and shows 16% disorder. In Ramachandran plot, all amino acids showed accumulation in the allowed region of protein conformation giving less steric hindrance and majority of amino acids fall under right handed α-helix region (Fig. 9). Wu et al. (2006) conducted a similar experiment on silicon uptake ability using hydroponics to compare the uptake among japonica (Kinmaze) and indica (DV85) rice under three different silicon concentrations ranging from 0.16-0.4mM. He observed that silicon uptake ability of the japonica rice is higher than that of indica rice on Si-deficient soil, regardless of the silicon concentration showed through a kinetic study and the time-course experiments. Similar results were shown by Deren et al. (1992) and Ma and Tamai (2003) for the silicon uptake studies. Ma et al. (2004 and 2007) found saturation level at higher silicon concentrations around 1.5-2.0mM, which is in consistent with the result of this experiment.

Yamaji et al. (2012) also performed a homology search, BLAST with the rice *Lsi1* cDNA sequence against the barley full-length cDNA library and detected the homology with the cDNA of *HvLsi6*. The open reading frame (ORF) of *HvLsi6* of barley consisted of 900 bps, encoding a peptide with 300 amino acids. He found that *HvLsi6*

exhibited 88.2% identity with rice *Lsi6* at the amino acid level. Similar to other Si-permeable channels, *HvLsi6* was reported to be characterized by two Asn-Pro-Ala motifs and a distinct aromatic/Arg selectivity filter, Gly, Ser, Gly, and Arg. Whereas, according to Ma and Yamaji (2006) the *Lsi1* gene is predicted to encode a membrane protein similar to aquaporins, the water channels proteins. The predicted amino acid sequence has six transmembrane domains and two Asn-Pro-Ala (NPA) motifs, which are well conserved in typical aquaporins. So, this research concentrated on allele mining of Si transporter gene in order to exploits the DNA sequences of one genotype to isolate useful alleles from related genotypes. A significant amount of natural allelic variation is known to exist for a given gene and such small polymorphic variation are implicated in evolutionary relationship between the gene and the trait in question. Thus this type of studies provides the raw materials for plant breeding programmes and translational genomic approaches (Latha et al. 2004).

Explanation to Figures:-

Figs. 1a-e:- (1a) Hydroponic culture of seven upland indica rice (*Oryza sativa*) varieties in different concentration of Si (0.5, 1.0, 1.5, 2.0mM) along with untreated control in Yoshida Nutrient solution; (1b) Comparison of seeding growth (both root and shoot) in var. Ghanteswari after 20d of treatment; (1c) Comparison of seedling growth in var. Lalat in different concentration of Si after 10d of treatment; (1d) Effect of Si on growth of var. Ghanteswari after 10d of treatment; (1e) Effect of Si on growth of var. Lalat after 10d of treatment.

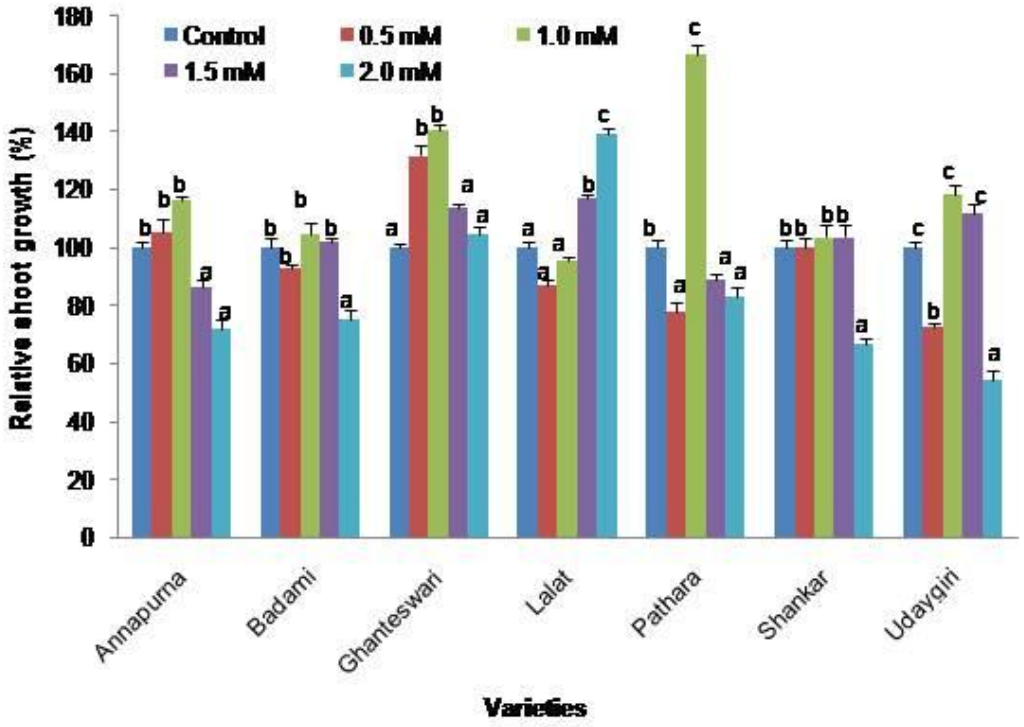

Fig. 2a

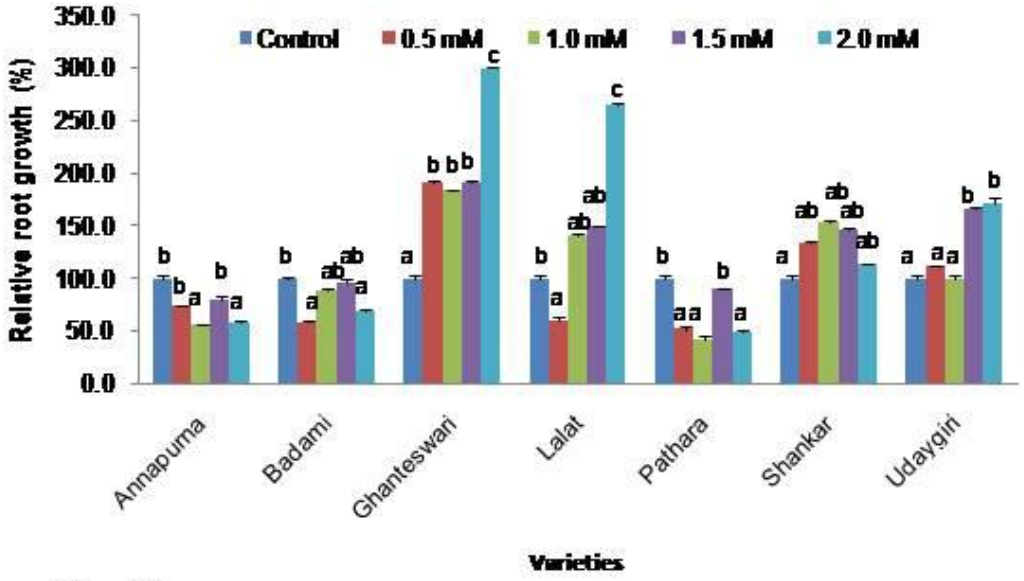

Fig. 2b

Figs. 2a-b:- Relative growth of seven varieties of rice seedling after 10 days of growth in different Si-concentrations (in %): (2a) Shoot growth; (2b) Root growth. Bar on the histogram represent ±S.E. and alphabets represent the statistical significance of value on the basis of Duncan's Multiple Range Test.

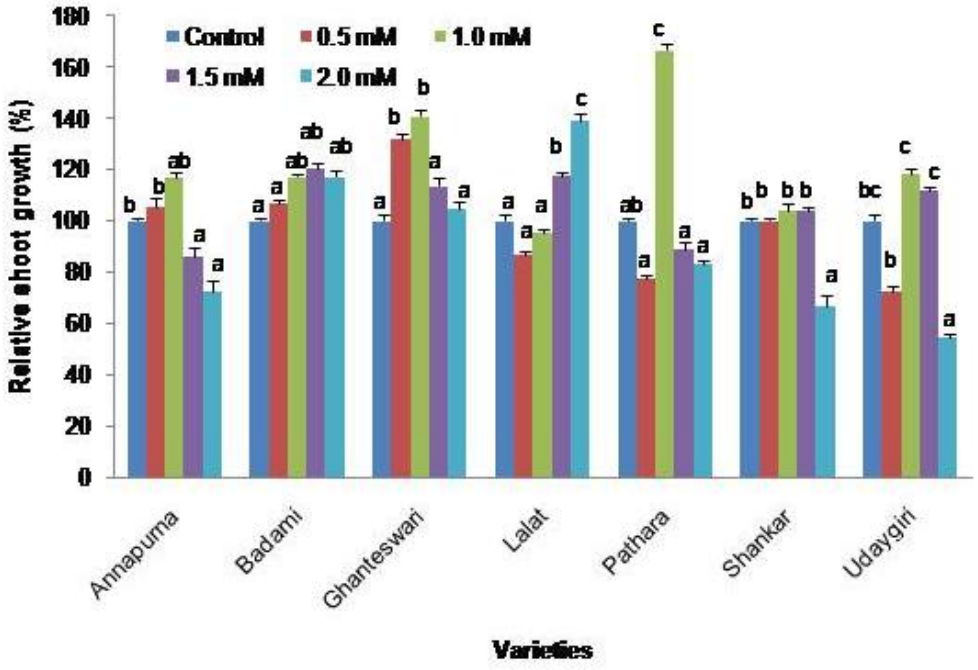

Fig. 3a

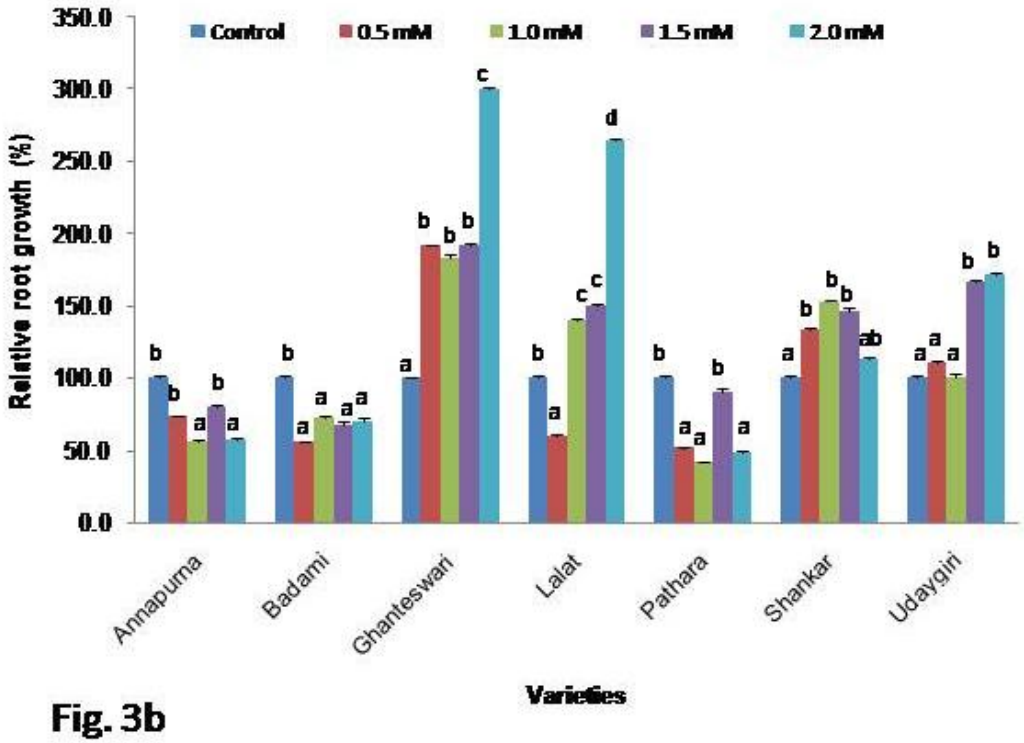

Fig. 3b

Figs. 3a-b:- Relative growth of seven varieties of rice seedling after 20 days of growth in different Si-concentrations (in %): (3a) Shoot growth; (3b) Root growth. Bar on the histogram represent ±S.E. and alphabets represent the statistical significance of value on the basis of Duncan's Multiple Range Test.

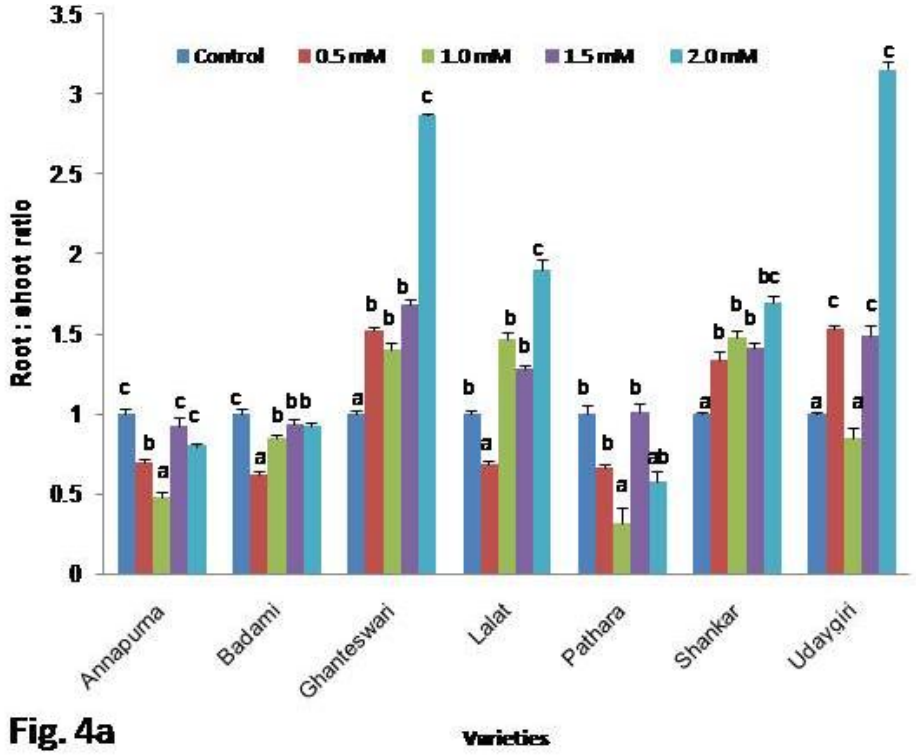

Fig. 4a

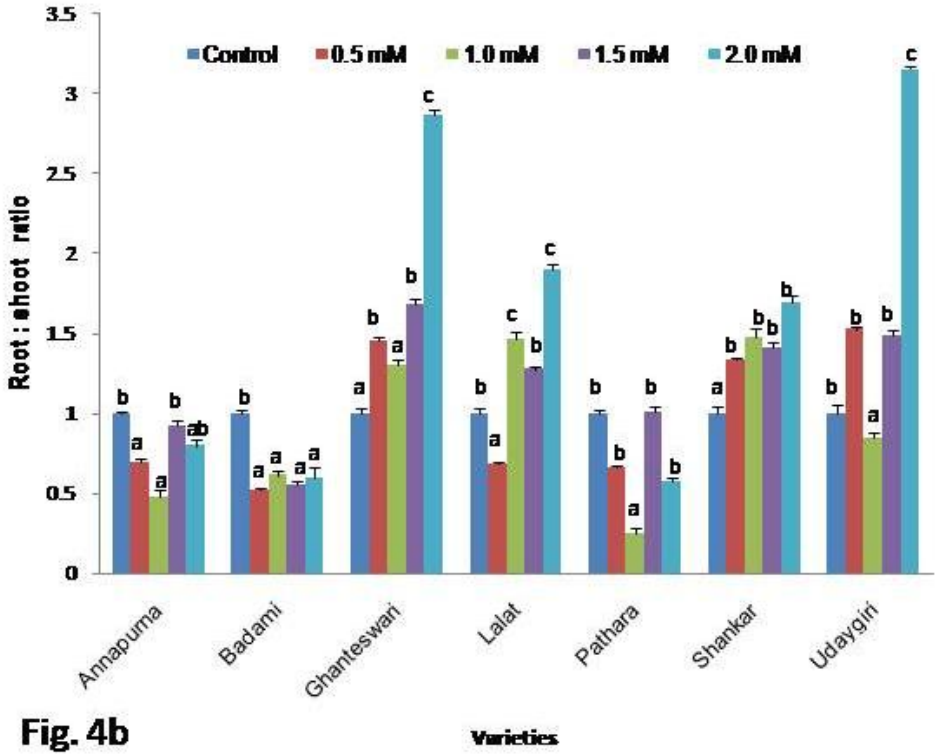

Fig. 4b

Figs. 4a-b:- Relative root: shoot ratio of seven varieties of rice seedling after 10 and 20 days of growth in different Si-concentrations: (4a) 10 days old seedling; (4b) 20 days old seedling. Bar on the histogram represent ±S.E. and alphabets represent the statistical significance of value on the basis of Duncan's Multiple Range Test.

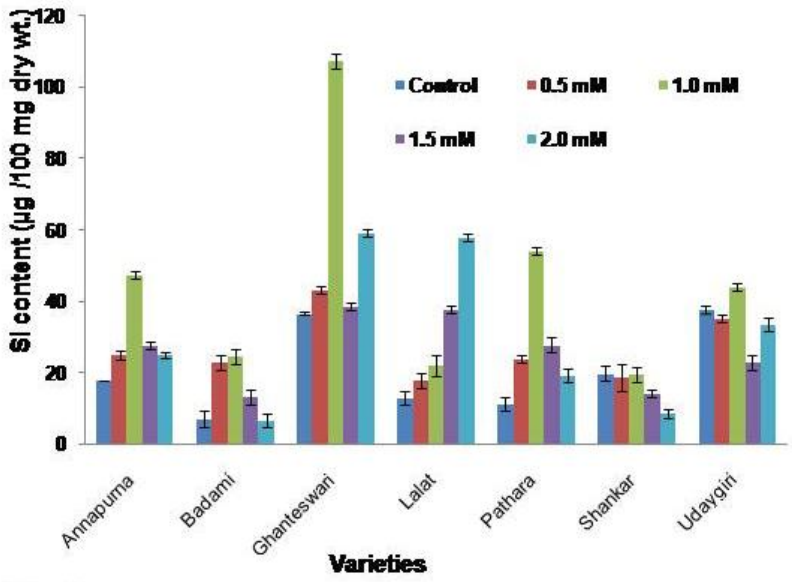

Fig. 5

Fig. 5:- Histogram representing the silicon content per gram dry weight of plant obtained by colorimetric molybdenum blue method in seven upland varieties of indica rice after 30 days of hydroponic culture growth at different concentration of Si treatment.

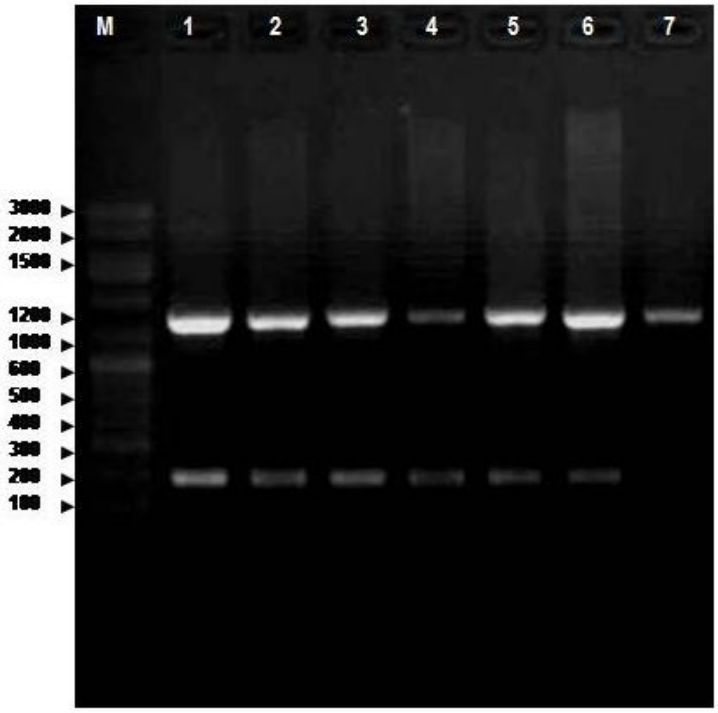

Fig. 6

Fig. 6:- Amplified product (~12000bp) of *Lsi2* gene with seven varieties of rice resolved in agarose gel.

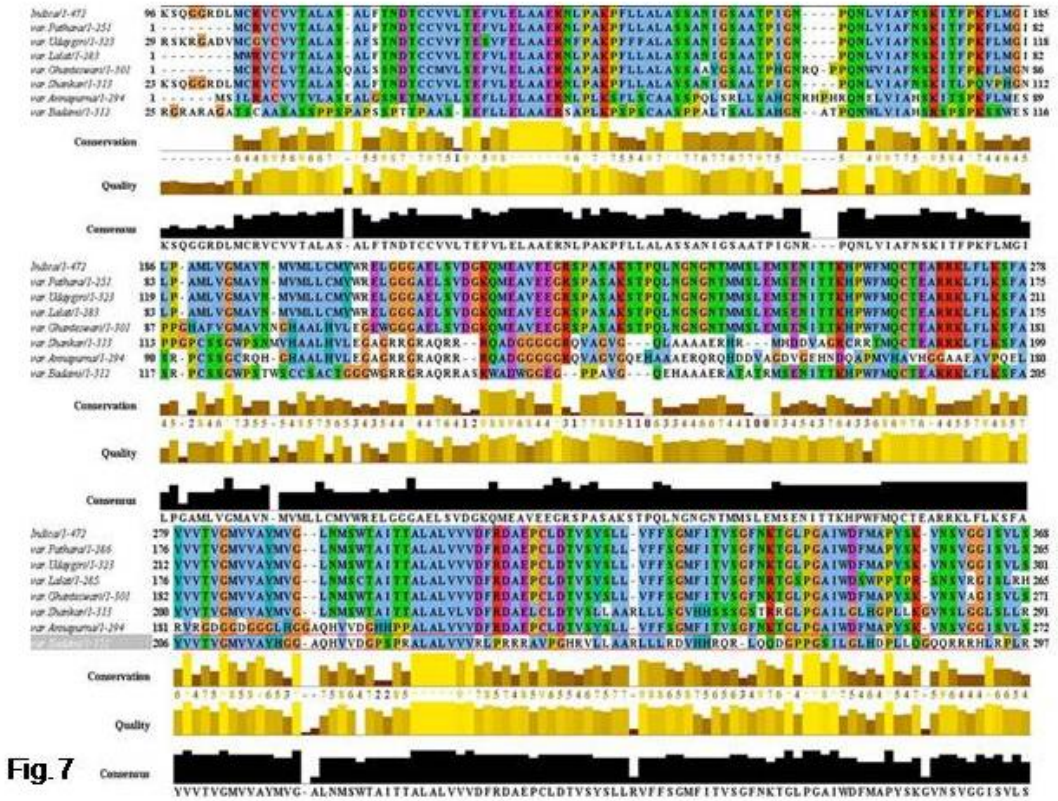

Fig. 7:- MSA of consensus sequences of *Oryza sativa* cv. Indica with the 7 varieties under investigation.

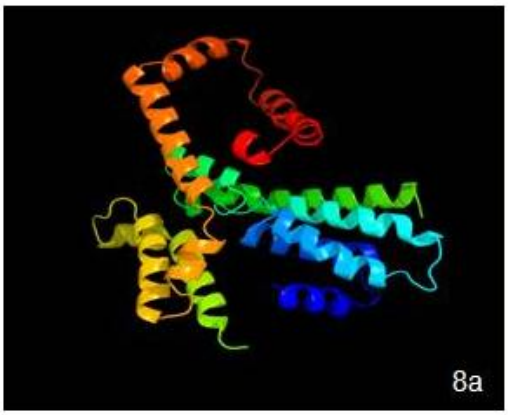

Fig. 8a:- Predicted 3D structure of Si-transporter gene in var. Ghanteswari (Phyre Server).

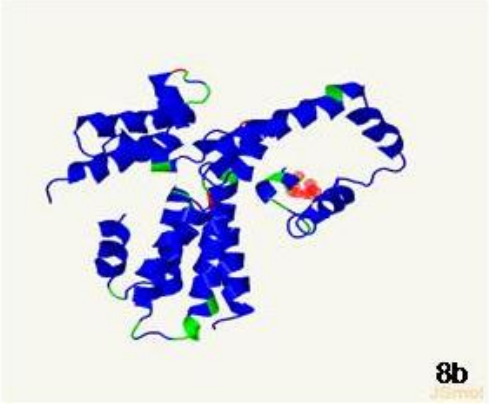

Fig. 8b:- 3D View of Ramachandran analysis of predicted protein structure.

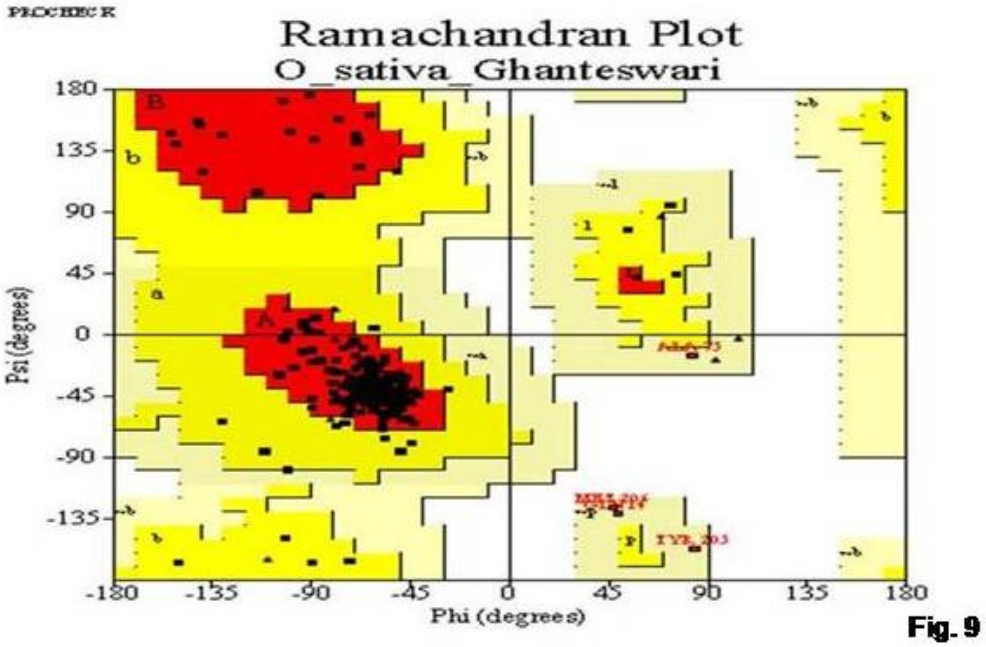

Fig. 9:- Ramachandran plot showing abundance of right handed α-helical amino-acids in predicted protein structure.

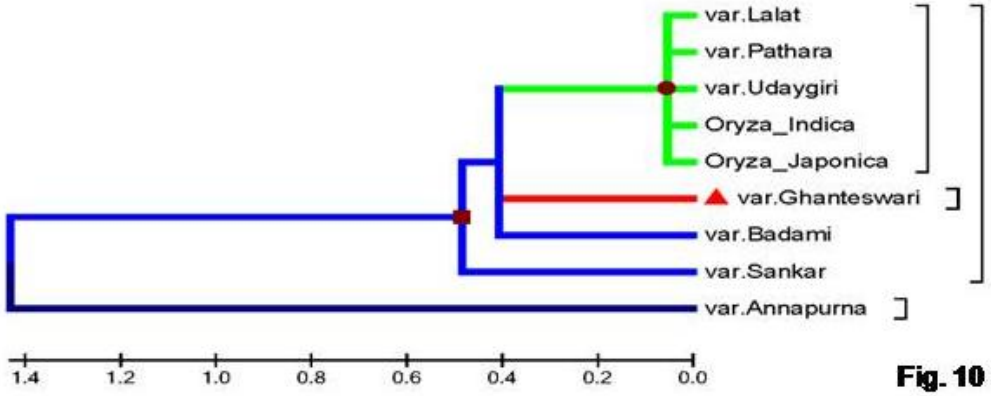

Fig. 10:- Dendogram shows the evolutionary trend of Si transporter gene (*Lsi2*) in seven studied varieties of rice alongwith *Oryza sativa* (cv. *indica* and cv. *Japonica*).

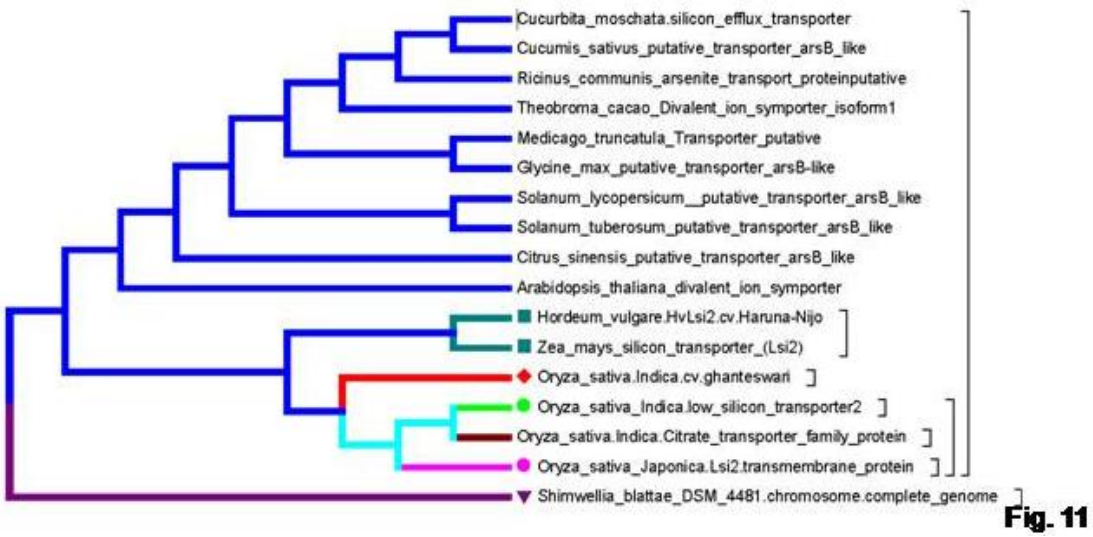

Fig. 11:- Phylogenetic tree constructed with sequences of rice *Lsi*2 genes of var. Ghanteswari and Si transporter genes reported in other crops, depicting the evolutionary trend.

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