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FORM 2

THE PATENTS ACT – 1970
(39 of 1970)

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THE PATENTS RULES, 2006

COMPLETE SPECIFICATION
(*See* section 10 and rule 13)

Iron fortification in maize plant and seeds via newly isolated siderophore producing *Pseudomonas* strain

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The following specification particularly describes and ascertains the nature of this invention and the manner in which it is to be performed:

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FIELD OF INVENTION:

The present invention relates to the field of fortification of nutrients to plants. The present invention more particularly relates to iron fortification in maize plant and seeds via newly isolated siderophore producing Pseudomonas strain.

BACKGROUND AND PRIOR ART:

Iron is one of the most important trace element and necessary as part of nutrition and physiology. It is required for production of RBC, being a part of haemoglobin transports oxygen from lung to all the cells of the body. According to the scientific studies, iron deficiency is the most common nutritional deficiency in the world, affecting about 25% of the global population, particularly young women and children. Major causes of iron deficiency are intake of food having less iron content, decreased absorption of iron (due to certain diseases or its presence in insoluble form) and increased requirements (during growth periods, blood loss, haemoglobinuria, iron sequestration). Humans derive iron from their every day diet, predominantly from plant foods and can be replenished in plants through the iron uptake from soil. Among the different plant food, major consumption is of cereals. In India, maize ranks fifth in area with annual production of around 10 million tones covering 6 million hectares. Maize as a crop has multiple uses but is chiefly grown for human and Livestock consumption. The seeds and cobs are used as basic raw material in various industries.

Publication No. CN104017759 (A) relates to high-yielding strains of Pseudomonas siderophore and applications. Produced siderophore of hydroxamic acid and citric acid type hybrid type of pathogenic bacteria and fungi antagonism siderophore can be used for the prevention and control of diseases in vegetable growing, and the ability to promote plant growth.

Publication No. WO2013090628 (A1) relates to microbial strains, compositions, and methods of use thereof to enhance the growth and/or yield of a plant. These bacteria excrete low molecular weight, high affinity ferric-chelating microbial cofactors that specifically enhance their acquisition of iron by binding to membrane bound siderophore receptors

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Publication No. US4479936 (A) relates to a method for protecting the growth of plants using an improved siderophore producing strain of pseudomonas putida particularly Pseudomonas putida NRRL-B-12,537 or an iron binding siderophore produced by the strain. The siderophore of the improved strain is significantly more active in repressing the growth of fungi.

Publication No. CN104531546 (A) relates to a strain of Pseudomonas putida SRPG-396 and its growth-promoting salt solution applied to the field of biotechnology in agriculture. The strain accession number CGMCC No.9397. This strain has dissolved inorganic phosphorus, auxin and siderophore function, can improve crop absorption of nitrogen, phosphorus, potassium, calcium, iron and other elements, reducing the absorption of sodium, itself has a good salt tolerance. can salt effectively alleviate soil salinization harm to crops caused by stress and promote the growth of crops under salt stress.

The article entitled "Growth promoting influence of siderophore-producing Pseudomonas strains GRP3A and PRS9 in maize (Zea mays L.) under iron limiting conditions" talks about maize seeds, bacterized with siderophore-producing pseudomonads with the goal to develop a system suitable for better iron uptake under iron-stressed conditions [Alok Sharma, B N Johri; Microbiological Research 158(3):243-8 · February 2003].

The article entitled "Plant perceptions of plant growth-promoting Pseudomonas" talks about pseudomonas for promoting plant growth by suppressing pathogenic micro-organisms, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance [Gail M Preston; Philos Trans R Soc Lond B Biol Sci. 2004 Jun 29; 359(1446): 907–918.].

The article entitled "Siderophores of Pseudomonas putida as an iron source for dicot and monocot plants" talks about uptake of iron by monocots and dicots with the help of siderophores of Pseudomonas [Bar-Ness, E., Chen, Y., Hadar, Y. et al. Plant Soil (1991) 130: 231].

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Thus, it is clear from the above cited articles that a lot of research has been done on plant growth promoting properties of Pseudomonas. However no efforts have been made on fortification of iron upto seed level of Maize.

Present invention aims to provide iron fortification in maize plant and seeds via newly isolated siderophore producing Pseudomonas strain.

OBJECTS OF THE INVENTION:

The principal objective of the present invention is to provide iron fortification in maize plant and seeds via newly isolated siderophore producing *Pseudomonas* strain.

Another object of the present invention is to provide iron fortification in maize plant and seeds using the *Pseudomonas*/ siderophore and not chelators like EDDHA and PSB.

Yet another object of the present invention is to provide transport of iron from soil to plant parts (stem, leaf and seed).

SUMMARY OF THE INVENTION:

In accordance with the said objectives, the present invention provides iron fortification in maize plant and seeds via newly isolated siderophore producing *Pseudomonas* strain. The seeds are processed and converted into needed preparations, flakes, grits and pops for human consumption. In the present invention, corn plants are grown in soil supplemented with siderophore producing *Pseudomonas* and these are observed to have higher iron content in stem, leaf and seed of maize plant than control. The AAS results reveal that the iron transported to the stem/ leaf/ seed increased considerably by 300%, 328%, 487.5% respectively when iron deficient soil is supplied with *Pseudomonas* bacterial culture.

BRIEF DESCRIPTION OF THE DRAWINGS

It is to be noted, however, that the appended drawings illustrate only typical embodiments of this invention and are therefore not to be considered for

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limiting of its scope, for the invention may admit to other equally effective embodiments.

Figure 1 represents the effect of different treatments on cob length, number of rows/cob and grains/ row of maize;

Figure 2 represents Iron transportation to different parts of maize plant (stem, leaf and seed) with the help of siderophore produced by *Pseudomonas* strains (RSP5 and RSP8);

Figure 3 represents schematic representation of interaction of siderophore (microbial) with iron (Fe^{+3}) to enhance the iron bioavailability ($Fe.^{+2}$) and transportation to different parts of plants.

DESCRIPTION OF THE PREFERRED EMBODIMENTS:

The present invention provides iron fortification in maize plant and seeds via newly isolated siderophore producing Pseudomonas strain. The seeds and cobs are used as basic raw material in various industries. The seeds are processed and converted into needed preparations, flakes, grits and pops for human consumption. In the present invention, corn plants are grown in soil supplemented with siderophore producing *Pseudomonas* and these are observed to have higher iron content in stem, leaf and seed of maize plant than control.

Quantification of siderophore is done for 14 isolates on Sodium Succinate Medium (SSM). SSM supplemented with Fe is inoculated with overnight grown culture of *Pseudomonas* and incubated for 24hr in incubator shaker. Sample is withdrawn after 24hrs from SSM and centrifuged at 8000rpm. Absorbance of supernatant is measured at 400nm.

The invention is described in detail with reference to the examples given below. The examples are provided just to illustrate the invention and therefore, should not be construed to limit the scope of the invention.

EXAMPLES

Example 1

Material and Method

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Isolation of siderophore producing *Pseudomonas*

Presumptive *Pseudomonas* were selected from lab culture isolated from Pine and Oak forest and agricultural land of Uttarakhand, India.

Quantification of siderophore production with Fe

Quantification of siderophore was done for 14 isolates on Sodium Succinate Medium (SSM). SSM supplemented with 20 μM Fe was inoculated with overnight grown culture of *Pseudomonas* and incubated for 24hr at 28°C in incubator shaker. Sample was withdrawn after 24hrs from SSM and centrifuged at 8000rpm for 8min. Absorbance of supernatant was measured at 400nm.

Siderophore Conc. = O.D x1500x1000 /16500 (mg/L) or (μg/ml)

Example 2

Plant growth experiment

On the basis of siderophore production RSP5 and RSP8 were selected to observe the iron transport in maize plant. Seeds of maize (*Zea mays* L.) were surface sterilized in 0.1% $HgCl_2$, followed by thorough washing with autoclaved distilled water. Bacterization of seed (seed load; 10^5 CFU per seed) was performed by using carboxy methyl cellulose (CMC) as matrix. This was followed by sowing seeds in pots (500 g soil volume/pot). Standard Hoagland's solution was used to irrigate the pots once a week; autoclaved double distilled water was used during rest of growth period. Standard Hoagland's solution supplemented with 20μM Fe was used to irrigate the pots for observation to stop siderophore production. The observations for root/ shoot length, Number of leaves, Length of corn, number of grains per corn, number of rows per corn, iron content in leaf, stem and seed and were taken after 70 days of sowing. Different combinations were designed in triplicates as: a) soil with Bacteria b) soil with Fe (20 μM); c) Soil with Bacteria and Fe (20 μM); d) Control: Without bacteria and Fe (20 μM). The results were analysed biometrically. Table 1 represents the effect of different bacterial treatments on the plant growth characteristics. The table represents the results of the plant growth experiment in presence and absence of 20 μM iron. The results were based on the shoot length, root length, cob length and number of grains per cob.

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Table 1: The effect of different bacterial treatments on the plant growth characteristics

Bacterial Treatments	Shoot length (cm) Mean ± Fe	Roots Length (cm) Mean ± Fe	Cob length (cm) Mean ± Fe	Number of grains Mean ± Fe
RSP5	156 ± 1.52	21.66 ±0.88	21.66 ± 0.881	395.66±44.29
RSP5 +Fe	149.66 ± 2.33	8.66 ±1.33	12 ± 1.52	156±12
RSP8	123.33 ± 3.33	12.33 ±4.09	18 ± 1.15	344±22.27
RSP8 +Fe	113.66 ± 0.88	8.33 ±1.66	8.66 ± 0.66	173.66±3.17
Fe	106.33 ± 7.57	9.33 ±0.66	8.33 ± 1.2	173.66±3.17
CONTROL	103.33 ± 4.80	7 ±0.57	10 ± 0.57	120.66±11.02

Example 3

Atomic absorption spectroscopy (AAS) of the plant parts (stem, leaf and seed)

Stem, leaf and seeds were collected separately for all the sample plants and labeled. Equal weight of each sample was weighed and burnt to ash in hot oven. Aqua regia was prepared taking HCl and HNO_3 in the ratio of 3:1. 15ml of aqua regia, 10 ml water and sample were mixed in separate beakers followed by boiling on hot plate. The mixture was boiled till the volume reduced to 8ml followed by filtration. The filtrate was then properly diluted and iron content was measured by AAS (Analytical Iena vario-6 cold Vapour- HG-AAS).

Example 4

Results:

Siderophore production by different Pseudomonas species has been widely studied and is found to be dependent on iron content present in soil. Our experimental findings revealed that *Pseudomonas* isolates RSP5 and RSP8 yield different amount of siderophore in iron deficient (≤ 20 μM) and iron sufficient (≥ 20 μM) soil.

RSP5 produces higher amount of siderophore than RSP8 in iron sufficient as well as iron deficient soil. The plant growth experiment suggested that siderophore produced by RSP5 solubilise more iron in soil and transport iron more efficiently to various plant parts of Maize (dietary food) than RSP8. The increased iron

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content in stem, leaf and seed of Maize will be beneficial for supplementation of dietary iron (non- heme) in human and animal food and feed.

Numerous modifications and adaptations of the system of the present invention will be apparent to those skilled in the art, and thus it is intended by the appended claims to cover all such modifications and adaptations which fall within the true spirit and scope of this invention.

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WE CLAIM:

1. A method for iron fortification in maize plant (*Zea mays* L.) and seeds via newly isolated siderophore producing *Pseudomonas* strain.

2. The method for iron fortification in maize plant and seeds as claimed in claim 1 wherein quantification of siderophore is done for 14 isolates on Sodium Succinate Medium (SSM) supplemented with 20 μM Fe and inoculated with overnight grown culture of *Pseudomonas,* incubated for 24hr at 28°C in incubator shaker, withdrawn after 24hrs from SSM and centrifuged at 8000rpm for 8min.

3. The method for iron fortification in maize plant and seeds as claimed in claim 1 wherein seeds of maize (*Zea mays* L.) are surface sterilized in 0.1% $HgCl_2$, followed by thorough washing with autoclaved distilled water.

4. The method for iron fortification in maize plant and seeds as claimed in claim 1 wherein bacterization of seed (seed load; 10^5 CFU per seed) is performed by using carboxy methyl cellulose (CMC) as matrix followed by sowing seeds in pots (500 g soil volume/pot).

5. The method for iron fortification in maize plant and seeds as claimed in claim 1 wherein standard Hoagland's solution is used to irrigate the pots once a week; autoclaved double distilled water is used during rest of growth period.

6. The method for iron fortification in maize plant and seeds as claimed in claim 1 wherein standard Hoagland's solution supplemented with 20μM Fe is used to irrigate the pots for observation to stop siderophore production.

7. The method for iron fortification in maize plant and seeds as claimed in claim 1 wherein *Pseudomonas* isolates RSP5 and RSP8 yield different amount of siderophore in iron deficient (≤ 20 μM) and iron sufficient (≥ 20 μM) soil.

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8. The method for iron fortification in maize plant and seeds as claimed in claim 1 wherein RSP5 produces higher amount of siderophore than RSP8 in iron sufficient as well as iron deficient soil.

9. The method for iron fortification in maize plant and seeds as claimed in claim 1 wherein siderophore produced by RSP5 solubilise more iron in soil and transport iron more efficiently to various plant parts of Maize (dietary food) than RSP8.

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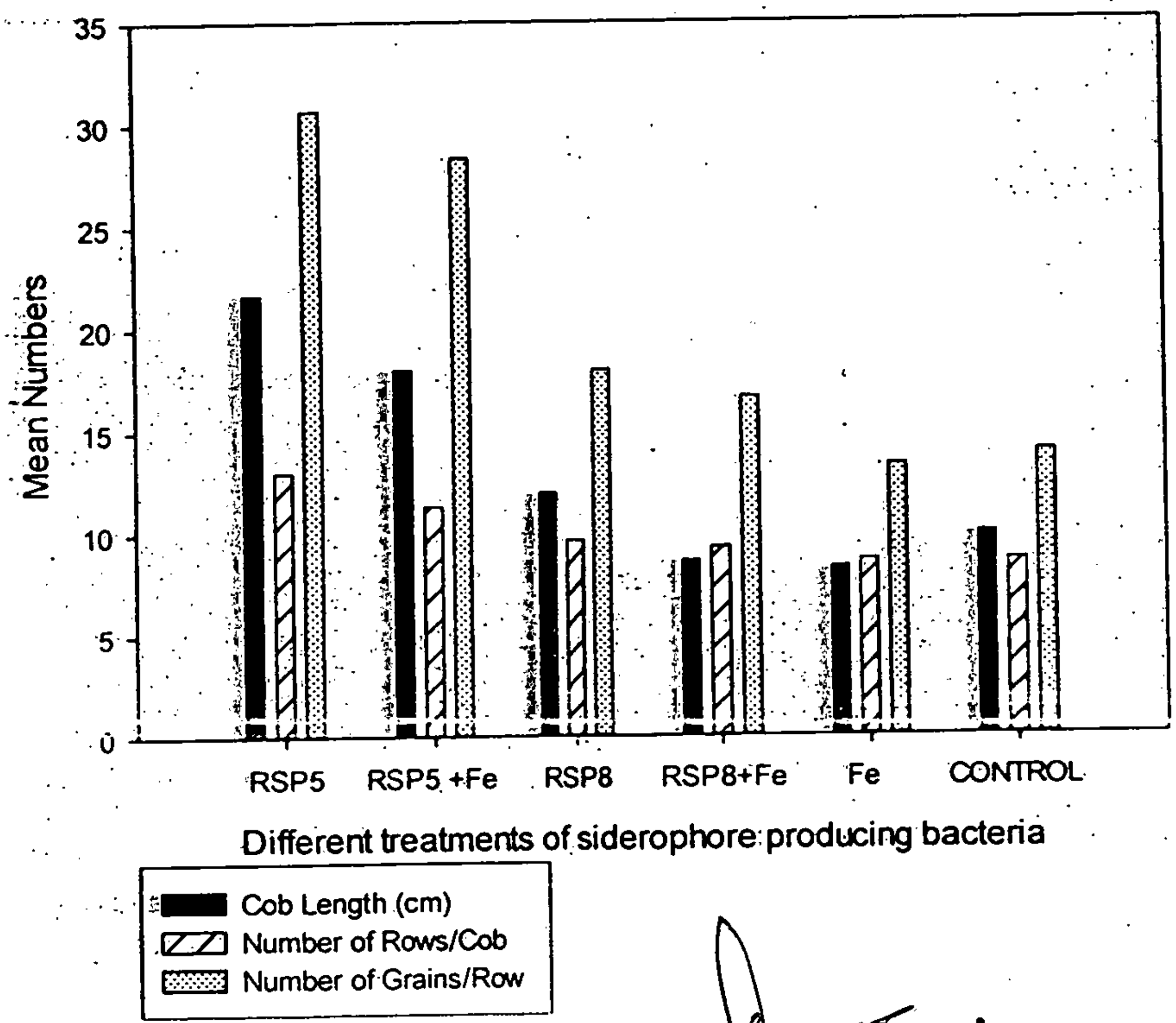

Figure 1

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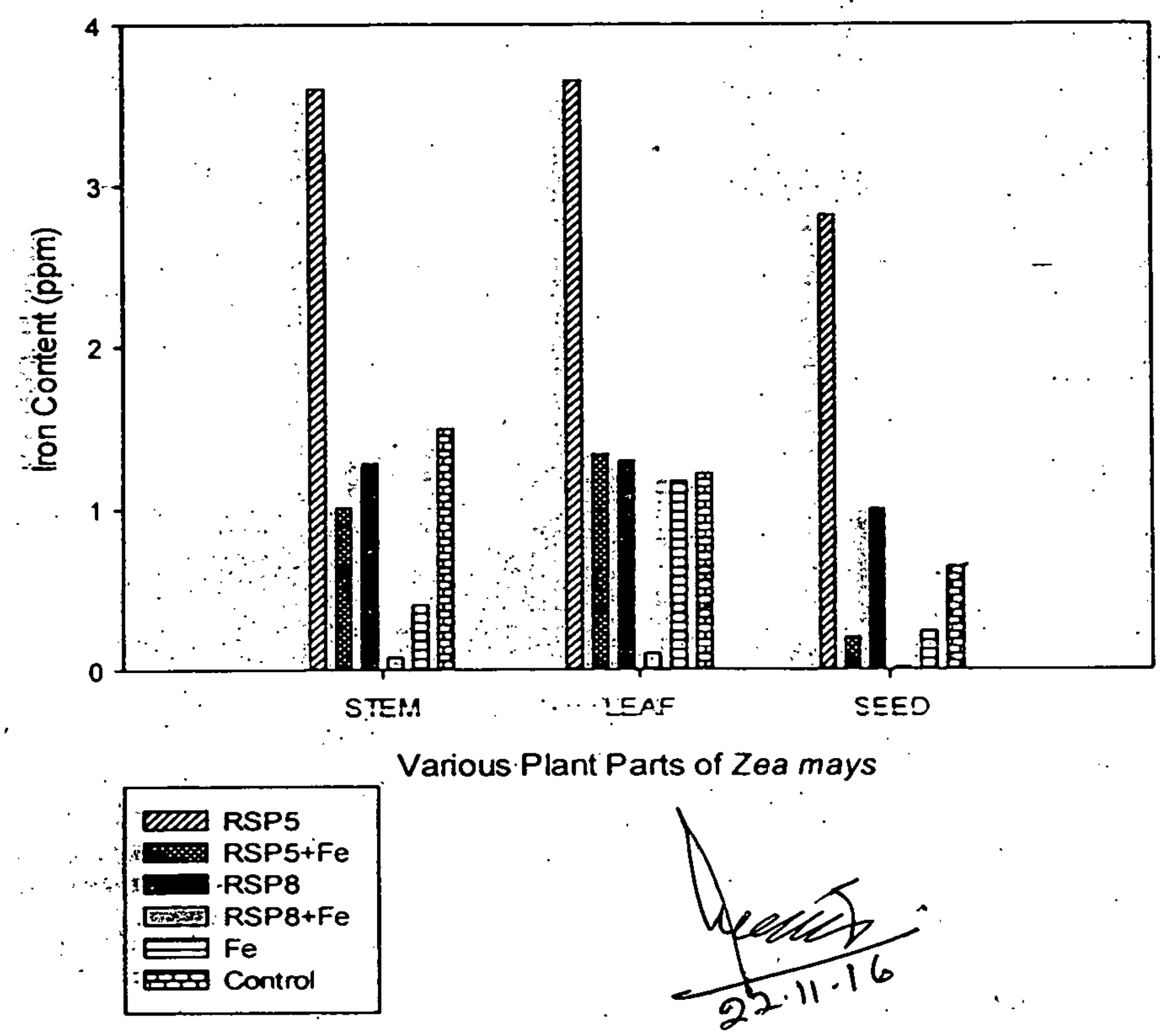

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Figure 2

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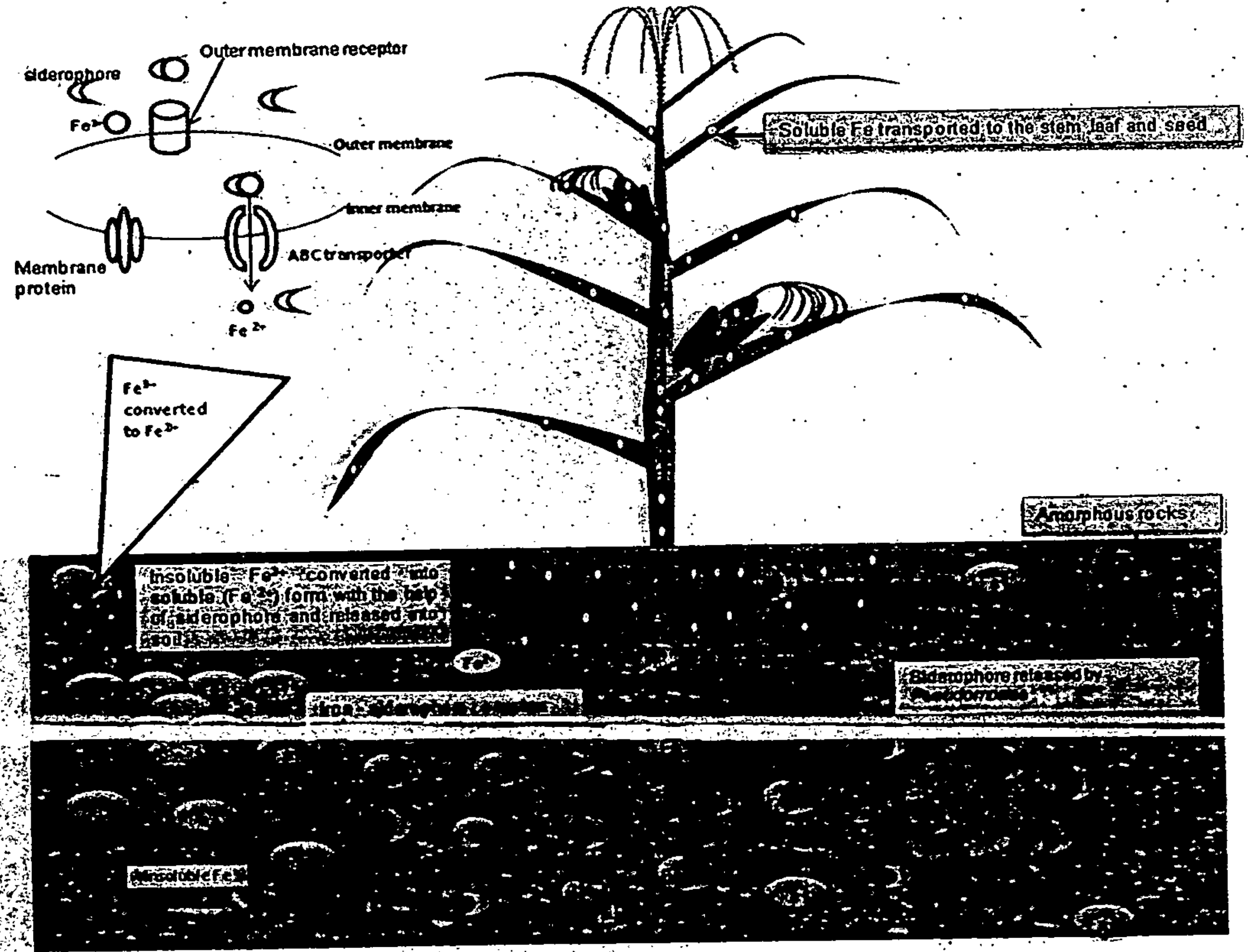

Figure 3

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ABSTRACT

Iron fortification in maize plant and seeds via newly isolated siderophore producing *Pseudomonas* strain

The present invention provides iron fortification in maize plant and seeds via newly isolated siderophore producing Pseudomonas strain. The seeds are processed and converted into needed preparations, flakes, grits and pops for human consumption. In the present invention corn plants are grown in soil supplemented with siderophore producing *Pseudomonas* and have higher iron content in stem, leaf and seed of maize plant than control. The AAS results reveal that the iron transported to the stem/ leaf/ seed increased considerably by 300%, 328%, 487.5% respectively when iron deficient soil is supplied with *Pseudomonas* bacterial culture. This fortification of iron in plants and seeds provides a good source of nutrition in food and fodder.

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