well as boll-rind thickness of *G. australe* was less when compared to domesticated *G. herbaceum* race *wightianum*. Further, seed index (Figure 2*f*), oil content and fibre length also fall in same line with boll observations, as considerable decrease in values of *G. australe* were noticed. In *G. herbaceum,* lint or fibre of nearly 21 mm length can be removed easily through ginning. On the contrary, in *G. australe* fibres of 6 mm length which are attached very tightly to the seed have been found to be unginnable (Figure 2*f*). The seed hairs are perfectly straight and radiate in all directions from the seed. Upon maturation, the capsules of *G. australe* flare widely, releasing the seeds to wind or gravity and enabling them to tumble quickly away with the first breeze. Hence, this species is most widely distributed in Australia. The colour of the fibre in *G. australe* was found to be brown as against white in *G. herbaceum*. Further, unmatchable uniformity exists in the fibres of this Australian species, indicating a source for fibre colour and uniformity.

The observations made by Stewart[6] indicated possibility of getting recombinants through back-cross for various traits discussed here. However, substantial efforts need to be made in terms of generating abundant back-cross populations and isolating glandless seed and glanded genotype without deleterious genes.

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Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus *Fomitopsis* sp. PS 102

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The field soil at Taegu, South Korea was screened for phosphate solubilizing fungal strains. Such strains were identified to be *Fomitopsis* sp. Phosphate solubilizing ability of *Fomitopsis* sp. PS 102 was studied on four different insoluble phosphates, viz. tricalcium phosphate, rock phosphate, aluminium phosphate and hydroxyapatite. Tricalcium phosphate was found to be solubilized maximally, while hydroxyapatite could not be solubilized by the isolated fungal strain. Further, the effect of salinity under *in vitro* conditions on the solubilization activity of rock phosphate was also observed. Presence of 1% NaCl enhanced the solubilization of rock phosphate by *Fomitopsis* sp. PS 102.

PHOSPHORUS is one of the major plant nutrients, second only to nitrogen in requirement. However, a greater part of soil phosphorus, approximately 95–99% is present in

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the form of insoluble phosphates and hence cannot be utilized by the plants[1]. To increase the availability of phosphorus for plants, large amounts of fertilizer are used on a regular basis. But after application, a large proportion of fertilizer phosphorus is quickly transferred to the insoluble form[2]. Therefore, very little percentage of the applied phosphorus is used, making continuous application necessary[3]. It has been reported that many soil fungi and bacteria can solubilize inorganic phosphates[4,5]. Phosphate solubilizing microorganisms (PSMs) play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers[6,7]. Application of PSMs in the field has been reported to increase crop yield[8,9]. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSMs[10–12]. Among PSMs, fungi perform better in acidic soil conditions[13]. Species of *Aspergillus*, *Penicillium*, *Curvularia* and yeast have been widely reported solubilizing various forms of inorganic phosphates[5,14,15]. Presently, rock phosphate is being chiefly employed to sustain soil phosphorus level in available form for plants[16]. Fungi have been reported to possess greater ability to solubilize rock phosphate than bacteria[17].

In the present study fungal strains having potential to solubilize insoluble phosphates were isolated. The fungal isolates were checked for the ability to solubilize different insoluble phosphates. Effect of salinity on the phosphate solubilizing activity of the isolated fungal strains, was also determined.

Fungal strains were isolated from the ground soil at Taegu, South Korea, and cultured on potato dextrose agar (PDA) media. Isolates were identified by their colony morphology, ability to form spores and microscopic study, according to Carranza-Morse and Gilbertson[18]. The isolates were checked for phosphate solubilizing ability on PDA (pH 6.5) supplemented with 0.5% (w/v) calcium phosphate. Formation of a clear halo around the fungal growth after 5 days of incubation indicates phosphate solubilizing ability. The strains were maintained on PDA slants at 4°C.

The strains were tested for the capacity to solubilize four common types of insoluble phosphates: rock phosphate, aluminium phosphate, tricalcium phosphate and hydroxyapatite. Quantitative estimation of phosphate solubilization activity of the fungal isolates was carried out in PD broth. The broth was amended with 0.5% (w/v) phosphates. Fifty millilitre broth was taken in Erlenmeyer flask, inoculated with fungal strains and incubated at 28°C for 20 days. Change in pH of the culture broth was recorded by Systronics pH meter, model number 324. Soluble phosphate in the broth was determined according to the method of Olsen and Sommers[19], up to 20 days and reported as particles per million (ppm) of free phosphate.

Effect of salinity on phosphate solubilization ability was also carried out in PD broth supplemented with 0.5% (w/v) rock phosphate. The composition (in per cent) of rock phosphate added to the broth was – P_2O_5, 34.23; Fe_2O_3, 0.52; Al_2O_3, 0.77; CaO, 50.21; SiO_2, 7.16; F, 3.23, and H_2O, 2.55, with ignition loss being 3.83%. NaCl was added in the broth in concentration from 0 (control) to 2% (w/v). The broth was inoculated with fungal strains and incubated at 28°C. Phosphate solubilization ability was determined as mentioned earlier. Change in pH of the culture broth was also recorded up to 20 days.

The fungal isolates were identified as *Fomitopsis* sp.[18]. The strains formed white-coloured colonies, which were also white on the reverse side. The texture of the colonies was powdery, with entire margins and velvety appearance. There was no spore formation recorded on PDA after prolonged incubation. The mycelia were septate. A clear halo was formed around the colonies after 5 days of incubation on PDA supplemented with calcium phosphate, indicating phosphate solubilizing ability of the fungal isolates (Figure 1). Further study on phosphate solubilization was carried out on strain *Fomitopsis* sp. PS 102.

The fungal strain *Fomitopsis* sp. PS 102 was able to solubilize rock phosphate, aluminium phosphate and tricalcium phosphate, but was almost unable to solubilize hydroxyapatite. The fungal strain discharged maximum amount of soluble phosphates in the culture broth with 1235 ppm concentration from tricalcium phosphate, after 12 days of incubation. The amount of soluble phosphate released by the fungal strain from rock phosphate and aluminium phosphate was 856 ppm and 1013 ppm, respectively, after 14 days of incubation. Irrespective of the insoluble phosphates taken, there was a decline in phosphate solubilization after 14 days of incubation. Choi *et al.*[20] reported soluble phosphate

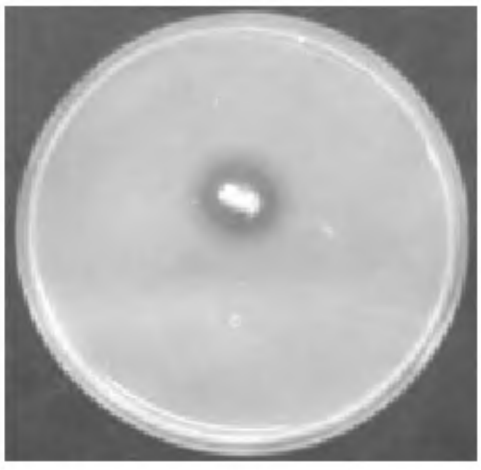

Figure 1. Phosphate solubilization by strain *Fomitopsis* sp. PS102, as indicated by hallow formation around the growth, on PDA medium with 0.5% calcium phosphate.

concentration of 1152 ppm from tricalcium phosphate and 565 ppm from rock phosphate in PD broth by *Penicillium* sp. GL-101, after 14 days of incubation. Phosphate solubilization declined or was stabilized after 14 days of incubation. Gaind and Gaur[21] reported that the drop in solubilization after a maximum value might be attributed to deficiency in nutrients in the culture medium.

Of all the inorganic phosphates, solubilization of rock phosphate was associated with steep decline in pH from 6.5 to 2.5, within 2 days of incubation. The pH remained at 2.5 up to 20 days, indicating the production of strong acids. Solubilization of the other insoluble phosphates was not accompanied with such decline in pH. Aluminium phosphate solubilization resulted in a very slight decline from 6.5 to 6.0, after 10 days of incubation. Aluminium phosphate was thus solubilized maximally at pH 6.0. In case of tricalcium phosphate there was an initial increase in pH from 6.5 to 7.0 after 6 days incubation followed by a decline up to 5.7.

Phosphate solubilization ability of the fungal strains was enhanced in the presence of NaCl and was maximum in the presence of 1% NaCl, with soluble phosphate concentration being 910 ppm in the broth compared to 845 ppm in control, after 12 days of incubation. Although phosphate solubilization declined after 12 days of incubation, it was higher in the presence of 1% NaCl compared to control even after further incubation, soluble phosphate being 858 and 800 ppm after 20 days, respectively. Johri *et al.*[22] also observed salt-induced phosphate solubilization. Phosphate solubilization in the presence of 1% NaCl was associated with lowering of pH. Up to 4 days there was a sharp decline in the pH from 6.5 to 4.8, associated with low phosphate solubilization activity. But with further incubation, phosphate solubilization increased with increase in pH. These results support earlier reports that phosphate solubilization is not necessarily accompanied with acid production[3,23]. Based on the end pH of the culture filtrate, Chhonkar and Subba Rao[24] have classified phosphate solubilizing fungi into two groups: those that produce acid and those that do not. According to this classification, strain *Fomitopsis* sp. PS 102 is a non-acid producer. The results also show that the change in pH is dependent upon the type of phosphate. Rock phosphate solubilization was associated with sharp decline in pH, while this was not the case with other phosphates taken in the study. Nahas[17] and Kucey *et al.*[7] showed that the solubilization of insoluble phosphates depends upon a multitude of factors, including decrease in pH, microorganism and the insoluble phosphate used. Leyval and Berthelin[25] observed that the production of acids during phosphate solubilization depends upon the microorganism and also on the type of insoluble phosphate used as the substrate. Nahas[17] also reported that fungi possess higher ability to solubilize rock phosphate and lower pH values were observed during rock phosphate solubilization than calcium phosphate, due to the acidic components of the former. Many studies have shown that the production of soluble phosphates is not necessarily correlated with acidity[26,27]. Bardiya and Gaur[28] suggested that the nature of organic acids produced is more important than the total acidity. However, further study is needed to confirm the mechanism involved in phosphate solubilization by the fungal isolate *Fomitopsis* sp. PS 102 with different substrates.

The results thus report the isolation of fungal strain *Fomitopsis* sp. PS 102 with the capability to solubilize different insoluble phosphates. The fungal isolate is capable of phosphate solubilization in saline conditions, *in vitro*. In fact, the phosphate solubilizing ability was enhanced in the presence of 1% NaCl. The strain *Fomitopsis* sp. PS 102 can thus be of great benefit in maintaining the available phosphate levels for crops in saline alkaline soils. A large fraction of land in arid and semi-arid regions is affected by salinity and in India alone about 7.5 million hectares of land are saline or alkaline[29]. The fungal strain *Fomitopsis* sp. PS 102 can thus be utilized in land reclamation of the saline regions along with biological nitrogen fixers. However, since conditions in the soil are far more complex, further study is required to assess the ability of the fungal isolate, *in vivo*.

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RGF–PCR: A technique to isolate different copies of a multi-copy gene

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Isolation of different copies of a multi-copy gene from the genome is a difficult process. To overcome the difficulty, a novel method known as 'Restricted Genomic Fraction–Polymerase Chain Reaction' (RGF–PCR) has been developed. RGF–PCR is a combination of fractionation of the restricted genome and PCR methods. The transposon Mariner-Like Elements (MLEs) occur as multi-copy genes in the silkworm, *Bombyx mori* genome. Using RGF–PCR method, isolation of different copies of the multi-copy MLE from different loci of the silkworm genome has been demonstrated. Polymorphic copies of MLEs have been isolated from restricted genomic fractions of silkworm, and the nature of polymorphism has been analysed and presented in this paper.

IT is often difficult to isolate different copies of multi-copy genes from the genome[1]. To understand the micro-heterogeneities and minor polymorphism of the multi-copy genes and gene families, it is essential to isolate and clone different copies of the genes. Using conventional method of PCR, multiple copies may be amplified from the genomic DNA in a single reaction. However, the amplified copies may not be distinguished as different copies of the gene[2]. Similarly, degenerate primers can also amplify multiple copies of the gene from the genome; however, the amplified copies may not be differentiated from one another as from different loci. To pick up different copies of a multi-copy gene, genomic or subgenomic libraries can be screened. However, it is a laborious and time-consuming process[3]. Following 'Restricted Genomic Fraction–Polymerase Chain Reaction' (RGF–PCR) strategy, different copies of a multi-copy gene can be easily isolated from different loci of the genome and the method is described in this article.

The transposons, Mariner-Like Elements (MLEs) are present in *Bombyx mori* genome as multi-copy genes. Four different types of MLEs, namely BmSMmar[4], Bmmar1[5], BmMLE[6] and BmoMar[7] are present in the silkworm genome. Among these four types, the copy number of Bmmar1 and BmMLE is about 2400 and 100/haploid genome, respectively[5,6], while BmSMmar

Table 1. Details of different primers used and the expected size of PCR products

Primer	Amplifying region	Expected PCR product size (bp)	Reference
BmSMmar124F BmSMmar265R	Internal region of BmSMmar transposon	444	4
MAR124F MAR276R	Internal region of all types of mariner transposons	470	4
Bmmar1-ITR	Full length Bmmar1 transposon	1300	5

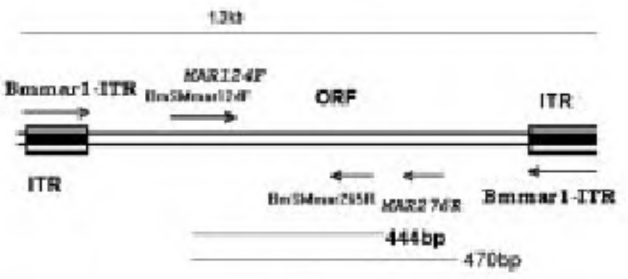

Figure 1. Schematic representation of the different primers used in RGF–PCR and their respective positions on a typical mariner-like transposable element. PCR amplifying regions and the fragment sizes are indicated. ITR, Inverted Terminal Repeat; ORF, Open Reading Frame. The primer sequences are as follows. MAR124F–5′TGGGTNCCNCAYGARYT 3′; MAR276R–5′ GGNGCNNARR TCNGG 3′; BmSMmar124F–5′ TGGGTGCCGCACCGAGTT 3′; BmSMmar265R–5′ GCCTAGCTCTGCGGCTTTC 3′; Bmmar1-ITR–5′ TCCTTACATATGAAATTAGCGTTTTGT 3′.

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