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Genotype dependant variation in arbuscular mycorrhizal colonization of tobacco (*Nicotiana tabacum*)

D V SUBHASHINI[1] and T G K MURTHY[2]

ICAR Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh 533 105

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Arbuscular mycorrhizal (AM) fungi (Phylum Glomeromycota) form ubiquitous symbiosis with most terrestrial plants by fungal species belonging to genera *Glomus*, *Entrophospora*, *Acaulospora*, *Gigaspora*, *Scutellospora*, *Archaeospora* and *Paraglomus*. These fungi impart many benefits to plants. Colonization of roots by AM fungi has been shown to improve growth and productivity of several field crops by increasing nutrient element uptake (Subhashini and Padmaja 2010). These fungi are also known to enhance crop growth and yield through enhanced tolerance to various biotic and abiotic stress factors and improving physical, chemical and fungi are also known to enhance crop growth and yield through enhanced tolerance to various biotic and abiotic stress factors and improving physical, chemical and biological properties of soil (Riedel *et al.* 2008). Research in the past years has proved that AM fungi can improve plant growth through increased uptake of phosphorus and other mineral nutrients, especially in low fertile soils (Hamel and Strullu 2006). In general, AM fungi improve the P uptake of their host plant especially under P limited conditions (Mohammed *et al*. 2004). AM fungi explore the soil more thoroughly and hence are able to locate and use the point source of P (Subhashini 2013b). The incidence of AM in tobacco has already been reported (Subhashini and Padmaja 2012). Based on the earlier reports that AM inoculation can increase the recovery of phosphatic fertilizer from soil by plants, field experiment was conducted at CTRI, Rajahmundry to find out the incidence of AM colonization and spore density in tobacco (*Nicotiana tabacum* L.) genotypes grown in vertisols. The present study is aimed to know whether the variation in AM symbiosis in tobacco crop is genotype dependant.

Key words: AM fungi, Genotype, Phosphorus, Spores, Tobacco

Fifty four accessions randomly selected from the germplasm collection of Central Tobacco Research Institute (CTRI), Rajahmundry during 2010 and 2011 was utilized for the study. Three replicate plants raised in clay loam soil (vertisols) having a pH 8.3, organic carbon 0.54% and available phosphorus 14.82 ppm. The soil had an indigenous AM fungal population of 23 spores/100 g of soil. Roots and rhizosphere soil samples (200 g soil in each sample) of three samples of each genotype (90 days after transplantation) were collected randomly, and brought to the laboratory for estimation of AM fungi association. Samples (0.5 g) of plant roots collected from the field were placed in perforated plastic holders and stored in cold water until they were processed. Samples were covered with 250 mL of 1.5 M KOH in a beaker and heated to 80°C in a fume hood for 30 min, then rinsed with water and briefly with dilute hydrochloric acid solution (5.0 mL conc. HCl in 200 mL H_2O), stirred and drained. Lactoglycerol trypan blue stain was dispensed into a beaker and heated to 80°C. Samples were placed in the stain for at least 30 min, then destained with lactoglycerol followed by two changes of tap water. The cleared and stained roots were spread in a 10 cm diameter Petri dish and observed under dissecting microscope for root colonization (Phillips and Hayman 1970). Mycorrhizal infection in the roots was expressed as the percentage of segments containing fungal structures like mycelium/fungal hyphae, arbuscules and vesicles (100 root segments per sample were evaluated for mycorrhizal infection).

$$\text{\% of mycorrhizal association} = \frac{\text{No. of mycorrhizal segments}}{\text{Total no. of segments screened}} \times 100$$

Individual AMF spores in soil showing hyphal connection were isolated using the modified wet sieving and decanting method of Daniels and Skipper (1982) and Gerdemann and Nicolson (1963) from the air-dried rhizosphere soil samples collected. Characterization of individual AMF spores was carried out after being subjected to morphogenetic and micrometric analysis based on their colour, diameter, shape, wall layers, surface content, hyphal colour, hyphal width and hyphal attachment with the wall. On this basis, dominant genera of AMF were categorized (Table 1 and 2), and the identification was made at species

[1]Principal Scientist (Agricultural Microbiology) (e mail: dv_subhashini@rediffmail.com); [2]Principal Scientist and Head i/c, Division of Crop Improvement (e mail: tgkmurthy@rediffmail.com)

Table 1 AM Fungi association in roots and spore propagules in soil for different FCV tobacco cultivars growing in their natural habitat in the vertisols

Tobacco genotypes	Soil pH	AMF structure		Mycorrhizal colonization (%)	AMF spores population per 100g soil	Phosphorus content (%)	AM fungal species
		Arbus-cule	Vesicle				
N 98	8.2	*	**	60.7	198	0.27	Gm,Gi, Gf
VT 1158	8.1	*	***	69.0	226	0.33	Ga, Gf, Gm
Kanaka Prabha	8.2		*	22.3	79	0.06	Gm
Dhanadhayi	8.2		*	26.3	185	0.23	Gc
Siri	8.0		**	54.3	182	0.14	
Harrison Special	8.2	*	***	62.7	185	0.24	Gi, Gf
Trupthi	8.1		*	40.0	172	0.18	Gi
Jayasri MR	8.2	*	**	54.0	54	0.37	Gc, Gm
Delcrest	8.1		*	11.0	74	0.06	Gm
Virginia Gold	8.2	*	***	61.1	183	0.23	Gm, Gi, Gf
CTRI Special	8.1	*	**	51.7	116	0.17	Gm, Gc
Sahyadri	8.3		*	17.3	76	0.14	Gc
Hicks	8.1	*	**	61.7	121	0.22	Gc, Gi
CM 12	8.0	*	**	61.2	170	0.28	Gm, Ga
Mcnair 12	8.2	*	**	63.3	106	0.21	Gf, Gi
CTRI Special-MR	8.2	*	***	74.0	210	0.40	Gc, Gi, Gm
Jayasri MR	8.1		**	52.3	107	0.13	Gc, Gm
16/103	8.2	**	***	70.7	195	0.27	Gc, Gi, Gm
FCV Special	8.3	*	***	61.3	183	0.19	Gm, Gi
Godavari Special	8.3	**	***	84.0	308	1.36	Gm, Gi, Gc
Ratna	8.1	*	***	63.6	209	0.28	Gc, Gi, Gf
Kanthi	8.1		*	18.3	79	0.06	Gi
Hema	8.1		*	26.6	77	0.12	Gm
Bhavya	8.0		**	46.3	105	0.16	Gf, Gc
Kanchan	8.1		**	58.3	147	0.23	Gc, Gm
Gauthami	8.2	*	***	61.6	170	0.22	Gm, Gc, Gf
Swarna	8.2	*	**	61.7	142	0.22	Gm, Ga
SEm ±	0.05			1.42	2.75	0.01	
CD (P=0.05)	0.15			3.92	7.63	0.03	
CV%	1.15			4.75	3.17	6.37	

* 1-30% infection level, Poor; ** 31-70% infection level, Moderate; ***above 70% infection level, Abundant; 0 absent. Each figure represents the mean of three replicates. Al = *Acaulospora laevis*; Ga = *Glomus aggregatum*; Gc = Glomus constrictum; Gf = Glomus fasciculatum; Gi = *Glomus intraradices*; Gm = *Glomus mosseae*; Gmi = *Glomus microcarpum*; Gs = *Glomus* species; Gsp = *Gigaspora* spp; Sc = *Sclerocystis* spp.

level with the help of relevant literature (Trappe 1982, Morton and Redecker 2001, Morton and Benny 1990, Schenck and Perez 1990, and Wu *et al*. 2002). For pH estimation, soil suspension (1:4 w/v) was prepared as described by Jackson (1973) and nutrient content of leaf, phosphorus by vanidomolybdate method . The data was analyzed statistically by analysis of variance.

FCV (Table 1) and non-FCV (Table 2) tobacco genotypes exhibited wide variation in percentage root colonization by AM fungi. Though all the FCV and non-FCV genotypes were grown in the same field under similar soil and environmental conditions and were receiving the same treatments, they exhibited differences in root colonization by AM fungi. This variation in percentage colonization could be ascribable to the interaction between host genotype and preference of AM species (Subhashini *et al*. 1988). The genetic make up and physiological need of the host plant may also contribute to the extent of colonization that one plant should have in its root system. The existence of genotype dependent variation in AM colonization has been reported earlier in crops such as barley (Tilak and Murthy 1987) and coconut (Thomas and Ghai 1987). Smith and Smith (1997), reviewing the literature and summarizing the AM types in different taxa of plants, proposed that the different AM structures have specialized roles in the transfer of inorganic nutrients and carbon between the partners, depending on the physiology of the symbiosis between different fungus and plant interfaces. The collected soil samples for estimation of AMF colonization and spore density belonged to different types of tobacco and had varying pH values. In the rhizosphere samples of FCV tobacco the pH range was from 8.0 to 8.3 (Table 1) and non-

Table 2 AM fungi association in roots and spore propagules in soil for different non-FCV tobacco cultivars growing in their natural habitat in the vertisols

Plant variety	Soil pH	AMF structure		Mycorrhizal colonization (%)	AMF spores population per 100g soil	Phosphorus content (%)	AM fungal species
		Arbus-cule	Vesicle				
Natu							
Natu Special	7.8	*	***	67.6	222	0.31	Ga, Gm, Gmi, Gi
Pyruvithanam	8.0	*	***	60.0	178	0.21	Gi, Gm
NG-73 Bhairavi	8.1	*	**	60.3	202	0.22	Gmi, Gi, Gm
Prabath	7.9	*	**	50.0	153	0.14	Gc, Gf
Vishwanath	8.2	*	***	64.6	210	0.28	Ga, Gc, Gf
Praba	8.0	**	***	81.6	237	0.54	Gm, Gc, Ga, Gf
Burley							
RV-31 Banket A1	8.1		**	50.3	160	0.13	Gf, Gc
BSRB-2	8.0		*	31.6	117	0.09	Ga
Burley	8.2		*	42.3	90	0.06	Gm.Gi,Gs
HDBRG	8.1	*	**	61.6	189	0.23	Gc, Ga, Gf
Lanka/Natu							
Prabath	8.1		**	61.0	191	0.28	Gi, Gm
Lanka Special	8.2		*	33.3	145	0.14	Gf
Chewing							
Vaishali Special	8.0	*	***	73.3	241	0.44	Ga, Gm, Gmi, Gi
Maragadham	7.9	*	***	67.6	173	0.26	Gf, Gc, Gm
Thangam	7.9	**	***	70.0	247	0.32	Ga, Gc, Gf
Vairam	8.0		*	26.6	93	0.03	Gi
Meenakshi	8.1		*	34.3	113	0.14	Gf
Abirami	8.2	*	***	79.0	265	0.55	Ga, Gm, Gi, Gf
Country Cheroot							
Sangami	7.9	*	***	71.6	230	0.46	Gm, Gi, Gc
Sendarapatty	7.9		**	56.3	190	0.25	Ga, Gm
Sendarapatty Special	8.0		*	21.6	93	0.13	Gc
Bhavani Special	8.1	*	**	51.6	139	0.12	Gf, Gm
Bidi							
Vedaganga NVD43	8.0		*	11.6	78	0.05	Gm
Cigar							
S 5	8.0	*	**	62.3	179	0.25	Gc, Gm, Gf
Krishna	8.0		**	51.6	109	0.17	Ga, Gc
Olor	8.1	**	***	69.0	197	0.31	Gm, Gc, Gf
Dixie Shade	8.1	**	***	70.0	217	0.33	Gc, Gf, Gmi
SEm ±	0.04			1.71	1.73	0.01	
CD (P=0.05)	0.12			4.73	4.79	0.03	
CV%	0.95			5.39	1.73	7.80	

*1-30% infection level, Poor; **31-70% infection level, Moderate; ***above 70% infection level, Abundant; 0 = absent. Each figure represents the mean of three replicates. Al = *Acaulospora laevis*; Ga = *Glomus aggregatum*; Gc = Glomus constrictum; Gf = Glomus fasciculatum; Gi = *Glomus intraradices*; Gm = *Glomus mosseae*; Gmi = *Glomus microcarpum*; Gs = *Glomus* species; Gsp = *Gigaspora* spp; Sc = *Sclerocystis* spp.

FCV tobacco the pH range was from 7.9 to 8.3 (Table 2). The mycorrhizal spore population in soil was higher in this pH range, and dominant genera of AMF were categorized as *Glomus***.** The average of mycorrhizal spore density ranged from 53-320 spores per 100g of dry rhizosphere soil of the FCV tobacco cultivars and the average of mycorrhizal spore density ranged from 81-271 spores per 100 g of dry rhizosphere soil of non-FCV tobacco cultivars. The results of root samples (Table 1 and Table 2) indicate the prevalence of AMF colonization in roots of several genotypes of FCV (Table 1) and non-FCV (Table 2) tobacco grown in vertisols. The average of mycorrhizal root colonization ranged from 15-90% in different genotypes of FCV and 10-85% in case of non FCV tobacco genotypes.A variety of spores were recovered from soils and root washings, mainly belonging to the genus *Glomus*. However, azygospore of *Acaulospora*

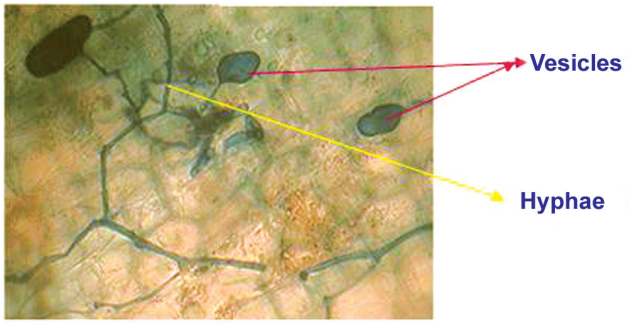

Fig 1 Microscopic image of Mycorrhizal structures in tobacco roots

and *Gigaspora*, and sporocarps of *Sclerocystis* were also recovered, though these were rare.

Most of the genotypes had prominent AMF association in the form of vesicular, arbuscular, and mycelium structure. The frequency of AMF infection and the percentage of root colonization varied among different genotypes of FCV and non – FCV tobacco host. AM fungal structures were found in all the cultivars of FCV and non – FCV tobacco. These fungal structures were frequently seen in the inner cortex of younger fine roots (Subhashini 2013a). The AM fungal hyphae were broader and thicker in the roots, and varied from genotype to genotype.

Vesicles and arbuscules were present in the roots of most of the FCV and non-FCV tobacco genotypes, and it was very difficult to identify the AM fungus species. To facilitate identification, the rhizosphere soil samples of all the 54 genotypes of both FCV and non-FCV were processed for isolating different fungal propagules. The rhizosphere soil samples of the entire plant contained AM fungal spores. Based on the spore characters, the following fungi were identified: Genus *Acaulospora*, six species of *Glomus*, viz. *Glomus aggregatum, Glomus constrictum, Glomus fasciculatum, Glomus intraradices, Glomus mosseae*, and *Glomus microcarpum*; one species of *Gigaspora*, one species of *Sclerocystis*.

G. mosseae seems to be the most predominant species, found in most of the rhizosphere soil samples of FCV tobacco cultivars (17); followed by *G. constrictum* (12), *G. intraradices* (11), G. *fasciculatum* (7), *G. aggregatum* (4), and *G. microcarpum* (4).The spore of *Acaulospora* was found in two FCV tobacco cultivars, VT-1158 and Gauthami while that of *Gigaspora* was found only in CTRI Special and the spore of *Sclerocystis* was found in the rhizosphere soil samples of Dhanadayi and one unidentified species of *Glomus* was found in Harrison Special (Table1).

G. mosseae seems to be the most predominant species, found in most of the rhizosphere soil samples of non-FCV tobacco (Table 2) cultivars too (15); followed by *G. constrictum* (13), *G. intraradices* (12), G. *fasciculatum* (7), *G. aggregatum* (4), and *G. microcarpum* (4).The spore of *Acaulospora* was found in two FCV tobacco cultivars, VT1158 and Gauthami while that of *Gigaspora* was found only in CTRI Special and the spore of *Sclerocystis* was found in the rhizosphere soil samples of Dhanadayi and one unidentified species of *Glomus* was found in Harrison Special (Table 1).

P content of the leaves increased apparently with increase in percent mycorrhizal colonization of the genotypes (both FCV and non-FCV tobacco) tested. The AM fungal infection consisted of fungal hyphae, vesicles, and arbuscules. The percentage of infection varied among the tobacco genotypes. No definite correlation could be established between spore and mycorrhizal root colonization. As multiplication of an endomycorrhiza depends on its association with plant roots, the number of its spores in soils is likely to differ, as shown in the present investigation. The prevalence of AM fungi suggests that the mycorrhizae may be of great importance for plant growth and development in tobacco growing soils, especially in vertisols where the crop is grown on residual soil moisture. The activity of mycorrhizae fungal population, in terms of root infection and the number of spores, has been shown to be greatly affected by soil conditions (Subhashini *et al*. 2011).

The present study clearly reveals that AM fungi are quite common in all the FCV and non-FCV tobacco genotypes examined, with species of *Glomus* found to be found to be dominant in most of the genotypes grown in vertisols. The *Glomus* species of AM fungi aids various types of tobacco in phosphorus uptake and growth in a sustainable manner.

SUMMARY

Different types of tobacco cultivars were examined for differences in arbuscular mycorrhizal colonization and spore density in rhizosphere samples. Fifty four genotypes of both FCV tobacco and non-FCV tobacco were tested across the field in vertisols. In case of FCV tobacco mycorrhizal colonization intensity ranged between 15 and 90% and spore density range was between 53 and 320 while pH range was from 8.0 to 8.3. In non-FCV tobacco the range of mycorrhizal colonization intensity was between 10-85% and spore density was between 81 and 271 and the pH range in the rhizosphere soil samples was between 7.9-8.3. The AM species associated with tobacco genotypes include *Acaulospora laevis*, *Glomus aggregatum, Glomus constrictum, Glomus fasciculatum*, *Glomus intraradices, Glomus mosseae*, *Glomus microcarpum*, *Glomus* species, *Gigaspora* spp and *Sclerocystis* spp. The dominant genera of AMF were categorized as *Glomus*. This supports the suggestion that VAM colonization and spread is dependant of the host genotype. Spore density differed significantly among the FCV and non-FCV genotypes and they also differed in their responses to P content of the leaf. Mycorrhizal significantly increased P content of the leaf. The utility of host genotype dependant differences in VAM symbiosis of tobacco germplasm is reported in the present study.

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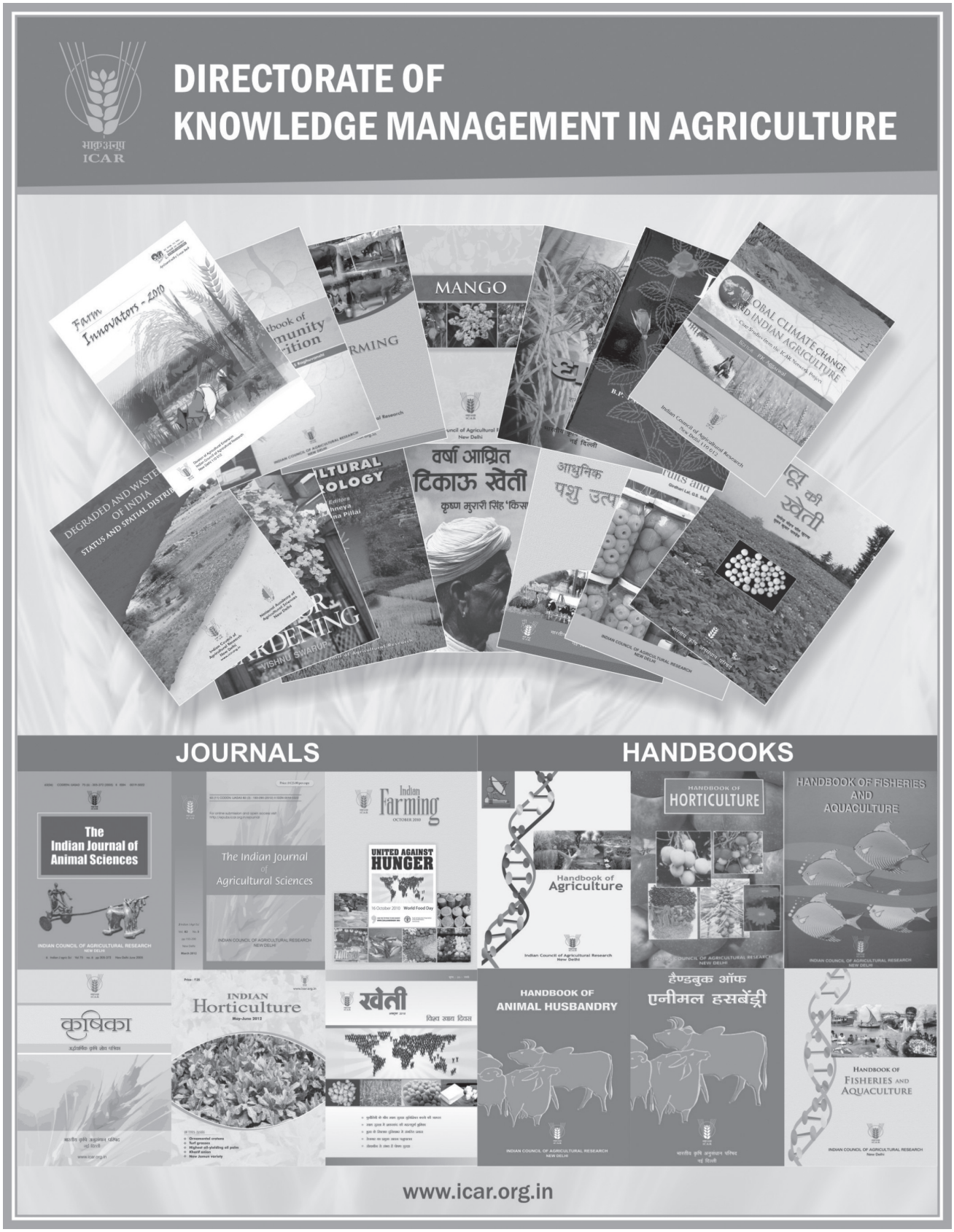
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