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Isotope-aided research in fruit and vegetable crops

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ABSTRACT

In the realm of newly-emerging horticultural enterprises, some of the tracer- or isotope-related applications are highlighted here. These include root activity studies on important fruit crops using soil injection of carrier-free ^{32}P, factors affecting spatial temporal root activity distribution of fruit crops, appropriate time and method of application to achieve maximum use efficiency in fruit and vegetable crops using ^{15}N- and ^{32}P-labelled fertilizers. A model for studying nutrient efficient cropping sequences and crop combinations in vegetable crops was also developed. Other uses of radiation techniques in plant nutrition are: mobilization of P from mother plant to sucker in 'Robusta' banana, contribution of exogenous fertilizer source to phosphorus availability in citrus, internal dilution technique for comparing sources not amenable to isotope labelling, determination of tree volume in fruit crops using isotope dilution technique, detection of unorthodox movement of nutrients upon direct feeding of nutrients to de-navelled banana bunch, monitoring internal dynamics of water in spongy-tissue affected 'Alphonso' mango fruits, investigations on source-sink relationship in mango and some vegetable crops, identification of high water use efficient types using $\nabla^{18}O$ values in onion, etc. Scope of using tracer techniques in horticultural research is highlighted.

Key words: Isotopes, root activity, ^{32}P, fertilizer use efficiency, ^{15}N, horticultural crops, tracer techniques

India has made tremendous advances in modern agriculture toward food and nutritional security. In recent years, agriculture and horticulture have assumed great importance mainly, to augment food and nutritional security and secondarily, to facilitate cost-effective, remunerative and eco-friendly enterprises with a vast export potential. To meet challenges in research and development, tracer techniques have made important contribution as these can be ingeniously applied to resolve issues not ordinarily possible through conventional techniques. Tracer- or isotope-aided research in agricultural crops has been done at various centres, but attention paid to horticultural crops is sporadic and largely restricted to a few fruit crops like mango, grape and banana. More concerted work was undertaken in the past three decades at Indian Institute of Horticultural Research, Bangalore, covering most tropical and sub-tropical fruit and vegetable crops. The Isotope Laboratory here was established in 1976 with financial assistance from USAID and DST and is an AERB approved Class II laboratory with an exclusive field facility, perhaps the best in the country. Most of the research has been carried out as field experiments. Salient achievement of this exemplary lead, along with contemporary research done is presented in this paper.

Nuclear techniques used in soil science and plant nutrition complement conventional techniques and provide unique information that other techniques cannot. Major applications of radiation techniques are: (i) Quantitative information generation on flow and fate of fertilizer in soil, and uptake of nutrients by plants, to develop efficient fertilizer management practices, (ii) Identification of the source of soil water and its availability to plants, (iii) Identification of sources of atmospheric/ soil carbon and estimation of their contribution, dynamics of photosynthates/assimilates to understand crop nutrition, (iv) Measurement of biological nitrogen fixation, and (v) Measurement of extent of soil erosion. Significant use of radiotracers in soil chemical and plant nutrition has been made. An ideal tracer is one which is physically, chemically and biologically indistinguishable from the tracer and which must not disturb the soil-water-plant system (Ramachandran *et al*, 2007). In other words, the 'isotope effect' should be practically absent. Radioactivity used in such studies should be low enough to not affect plants (the experimental material) or experimenters. Normally, these criteria are easily met in practical situations of soil/crop research.

The mainstay of tracer studies used in plants and soils is the principle of isotope dilution, propounded by G. Hevesy and R. Hobbie in 1932, the term being first introduced by D. Rittenberg and G.L. Foster in 1940. Dilution of the radioactive isotope by its non-radioactive counterpart results in reduction of specific activity (defined as activity per unit volume or mass) in a conserved manner, proportional to the original specific activity and amount of the analyte (Fasset, 1995). When stable isotopes are used, diluted natural isotopic ratio is related to concentration by the amount and isotopic composition of the separated isotope that was added. The principal limitation, however, is availability of suitable spike or tracers. The half-life must be long enough for sufficient activity to be available during the analysis for good counting statistics. Other important advantage of tracer techniques is the highly sensitive detection possible when appropriate instruments are installed in an isotope laboratory.

Root activity studies in fruit crops

Efficient use of fertilizers is a function of chemical availability of the nutrient and volume of soil explored by the roots. Information on root activity is useful in standardizing time and method of fertilizer application, irrigation, planting distance and other cultivation practices (Bojappa and Singh, 1973). The pattern of root activity distribution obtained using radioactive ^{32}P compared well with the actual (Purohit and Mukherjee, 1974). Root activity studies in important fruit crops were carried out on a Typic Haplustalf soil having textural horizon (B_t) at 20cm depth. Therefore, deeper layers were of hard to very hard dry consistency, blocky structure and slow infiltration rate, all of which increase mechanical resistance to root growth in drier months. Soil injection of carrier-free ^{32}P was used to study root activity distribution in important fruit crops under rain-fed conditions. The unique advantage of ^{32}P as a tracer for root activity studies is its relative immobility in soil (making location of isotope application effective for measurement of root activity) and its high mobility within the plant once absorbed, such that the tracer becomes homogenously distributed within the plant in a couple of weeks after application. Its convenient half-life of ^{32}P (14.3 days) makes it possible for four quarterly injections when root activity is required to be monitored during annual growth cycle of the perennial plants. Its amenability to Cerenkov counting which make detection convenient, is yet another advantage. Results indicated that citrus varieties [kinnow mandarin (Iyengar, 1983); *kagzi* lime (Iyengar and Keshava Murthy, 1987); Coorg mandarin

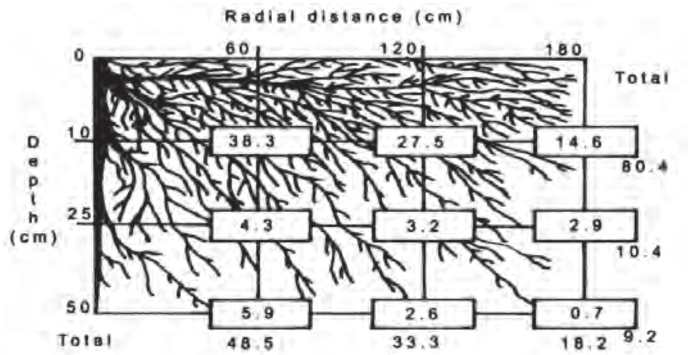

Fig 1. Surface-oriented root activity distribution (Example: 6 year old Coorg mandarin, Iyengar and Keshava Murthy,. 1988b)

Iyengar *et el*, 1988; Table 3, Fig. 1); and Sweet orange cv. Mosambi (Iyengar and Shivananda, 1990a)] were shallow-rooted. In these crops, fertilizer applied at a shallow depth can be optimally absorbed, as also drip irrigation/ fertigation can be efficacious. Both grape varieties viz., 'Anab-e-Shahi' and 'Thompson Seedless' (Prakash, 1987; Iyengar *et al*, 1989 and Iyengar and Shivananda, 1990b), mango cv. 'Alphonso' (Bojappa and Singh, 1973; Kotur *et al*, 1997), guava cv. 'Arka Mridula' (Kotur *et al*, 1998, Fig. 2), 'Ganesh' pomegranate (Kotur and Keshava Murthy, 2003b) and 'Cricket Ball' sapota (Kotur, 2005) were deep-rooted in nature. These deep-rooted crops utilize native nutrients effectively from the entire rooting volume but may show limited utilization of (i) nutrients applied at shallow depth and (ii) irrigation water. Spread of active roots in fruit trees was uniform during North-East monsoon due to uniformly moist soil-water regime. During summer, the active roots moved towards soil surface and away from trunk towards the periphery of drip-circle. Intensity of root activity (total radioactivity recovered during the season) was highest during South-West monsoon due to high volumetric soil-

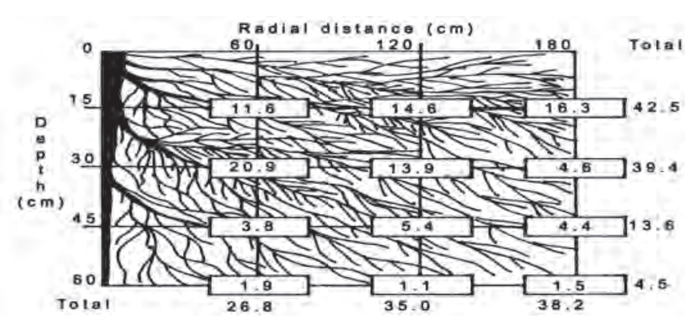

Fig 2. Deep-rooted root activity distribution (eg., 6-year old 'Arka Mridula' Guava; Kotur *et al*, 1998)

moisture, and decreased during winter as rains receded. Least intensity of root activity was observed during summer from depletion of soil moisture. Generally, a period of high root activity alternated with shoot growth except in guava, in which, both the periods of high root activity and shoot activity coincided. However, 'Arka Mridula' guava was an exception (Kotur *et al,* 1998). In 'Surya' papaya (Kotur and Keshava Murthy, 2001), one year after planting, active roots spread uniformly to a depth of 45cm and to a radial distance of 100cm from the trunk (Fig 3). Similarly, in 'Robusta' banana at the shooting stage, active roots extended up to 75cm lateral distance and 45cm depth, leading to fairly uniform root activity distribution in the entire rooting volume (Kotur *et al*, 2011). Thus, both banana and papaya grown under irrigated condition were found to utilize fully rooting volume both in terms of radial distance (subject to spacing) and depth (45cm) uniformly, owing to the congenial soil physical conditions round the year. Thereby, these crops can be exhaustive on native soil nutrients.

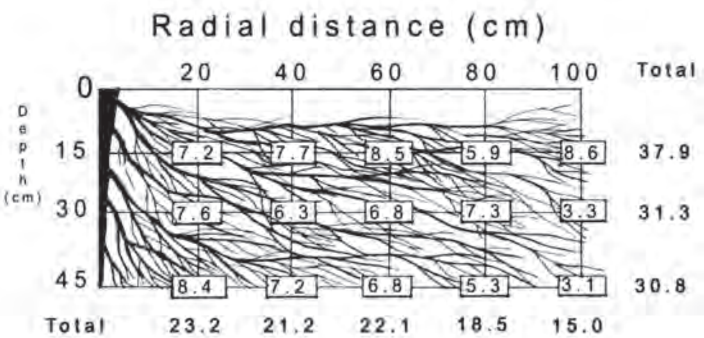

Fig 3. Uniform root activity distribution (eg., one-year old 'Surya' papaya; Kotur and Keshava Murthy, 2001)

In the second phase of root activity studies, effect of various production practices on were evaluated since tracer techniques are sensitive. Salient findings are:

(i) In 'Ganesh' pomegranate, type of planting material used had significant impact on root activity pattern. Air-layers showed predominantly surface-oriented root system by confining 98% of active roots up to 50cm depth, while seedlings extended roots up to 100cm depth. Conversely, air-layers extended roots to a wider distance (75cm) compared to seedlings (50cm). Seedlings showed better anchorage and were amenable to closer spacing (high planting density) than air-layers (Kotur and Keshava Murthy, 2004a).

(ii) In 'Arka Mridula' guava trees aged 16 years, cultural practices used for maintaining plant bed had a distinct influence on root activity. Intensity of root activity was higher under polythene mulch, followed by green manure mulch and non-cultivation. The dept-total of root activity was substantially higher at 160cm distance under black polythene mulch during winter (50%) and under green manure mulch (41%). In the rest of the treatments and seasons, 31-37% of active roots were present at this distance (Kotur, 2007a).

(iii) In 'Arka Mridula' guava, with increasing age, active roots extended predominantly sideways than deep owing to anisotropism of the soil. In 7-year old trees, bulk of the active roots extended up to 150cm radial distance, while, in 16-year old trees the roots extended to a distance of 240cm. As a result, to achieve high fertilizer use efficiency, the circular band of superphosphate had to be shifted from 100-160cm in young trees to a much farther distance of 130-190cm in older trees (Kotur, 2007b).

(iv) Assessment of root activity distribution in *annona* as influenced by rootstock-scion interaction showed that, in general, seedlings of both *Annona squamosa* and *A. reticulata* had higher intensity of root activity than when these were grafted with 'Arka Sahan' scion. Root activity distribution in *A. reticulata* trees closely conformed to that in *A. squamosa*. Grafts of both the species showed relatively uniform distribution of active roots throughout the year compared to seedlings (which may promote better anchorage, drought tolerance and utilization of native soil moisture and nutrients from the rooting volume) (Kotur, 2009).

(v) Application of Paclobutrazol, a growth retardant, to 20-year old 'Alphonso' mango trees to regularize fruit bearing showed reduced intensity of root activity. Active roots tended to bunch close to the soil surface and tree trunk compared to 'Control' trees (Kotur, 2006). This effect was pronounced during seasons of high soil moisture (Kotur, 2012). This calls for rationalization of fertilizer placement in such trees to optimize fertilizer use and to prevent decline.

Selection of uniform fruit trees in studies such as these is an important pre-requisite to contain coefficient of variation. However, good comparison of the pattern of root activity distribution from tracer studies with actual distribution using excavation studies, has been observed (Purohit and Mukherjee 1974). Zapata (1990) listed several sources of error like spatial variability of soil across the plantation, plant variability related to genetic origin, sampling factors (type, size, time, etc.), injection factors related to unequal probability of contact between root and the isotope applied.

Therefore, it is essential to characterize and earmark a fairly uniform land for root activity studies, and to interpret results keeping soil characteristics in view. When soil depth, shallow water-table and physical properties are not a constraint allowing for free growth of roots (as, perhaps, in alluvial soils), rooting pattern in any plant may be as characteristic and unique as is its shoot canopy. A pilot study is a must to standardize isotope to be applied to each tree (to obtain substantial counts during detection of the tracer) geometry of the location of points of injection for effective evaluation of rooting volume and, procedure and frequency of sampling, is essential before embanking on detailed studies. Once these protocols are standardized, root activity distribution of a large number of crops can be studied quickly, efficiently and cost-effectively.

Appropriate time and placement of fertilizer in fruit crops

Fertilizers are, perhaps, the single costliest item in cost of cultivation (accounting for 30-40% of the cost). Growers use fertilizers in ample measure notwithstanding their cost, for obtaining high yields. However, indiscriminate use of mineral fertilizers also leads to pollution of soil, water and the atmosphere. Fertilizer use aims at supplying required nutrients to a crop and not to the soil. Fertilizer use efficiency is a quantitative measure of actual uptake of the fertilizer nutrient by a plant/crop in relation to the amount of nutrient added to soil. It is commonly expressed as:

$$\text{Utilization of added fertilizer (\%)} = \frac{\text{Amount of nutrient in plant derived from fertilizer}}{\text{Amount of nutrient applied as fertilizer}} \times 100$$

It is the key to achieve highest possible yield with a minimum fertilizer input. In other words, efficient fertilizer management is most cost-effective and eco-friendly.

Isotopic techniques are superior to conventional methods as they give a direct and quantitative measure of isotope-labelled fertilizer nutrients utilized by the crop, quite independent of the native nutrients present in soil. As a result, different fertilizer practices like placement, timing, sources, etc., can be evaluated efficiently. The most important parameters determined in this technique is the **f**raction (of the nutrient in the plant) **d**erived **f**rom (labelled) **f**ertilizer is **fdff**. This fraction is expressed as percentage, i.e., fdff (%) as follows:

In the case of ^{15}N studies,

$$\text{Ndff (\%)} = \frac{\text{Percent } ^{15}\text{N atom excess in plant sample}}{\text{Percent } ^{15}\text{N atom excess in labelled fertilizer}} \times 100$$

And, in the case of ^{32}P studies,

$$\text{Pdff (\%)} = \frac{\text{Specific activity of } ^{32}\text{P in plant sample}}{\text{Specific activity of } ^{32}\text{P in labelled fertilizer}} \times 100$$

Labelling or enriching a fertilizer with the desired isotope is indispensable for this work, as, use of the labelled soil is not satisfactory. With phasing out of ^{32}P-labelling facility for superphosphate by Board of Radiation and Isotope Technology (BRIT), Mumbai, in 2003, the National Facility for labelling superphosphate with ^{32}P was established at the Indian Institute of Horticultural Research, Bangalore in the Isotope Laboratory, as a joint venture with BRIT in 2005, and has been functional ever since.

Fertilizer use efficiency in relation to application time and placement method was studied in some fruit crops raised on red sandy-loam (Typic Haplsutalf) weakly acidic in reaction, having low cation exchange capacity under semi-arid tropic conditions, using ^{32}P-labelled superphosphate and ^{15}N-labelled ammonium sulphate. The fertilizers were placed in circular bands around the plant during different seasons. N and P derived from fertilizer (Ndff and Pdff) were determined to monitor differences in absorption due to time of application and placement method. It was found that in eight year old plants of Sweet orange (Iyengar and Keshava Murthy, 1988a) and Coorg mandarin (Iyengar and Keshava Murthy, 1989), there was better absorption of fertilizer during late rainy season with the fertilizer placed in a circular band 105cm to 135cm from the trunk (Fig. 4, Table 1). A comparison of nine year old scion

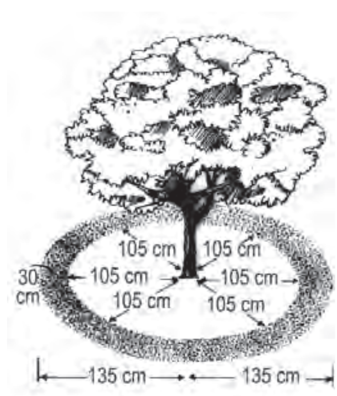

Fig 4. Proper placement of fertilizers in 8 year old citrus plants (Iyengar *et al*, 1996)

Cultivars, viz., Italian lemon (*Citrus latifolia* Tanaka) and Seedless lime (*C. limon* Birm.) showed that the dwarfing rootstock 'Trifoliate orange' (*Poncirus trifoliata*) resulted in highest Pdff, while, Rangpur lime (*C. limonia* Osbeck.) and Cleopatra mandarin (*C. reshni* Tanaka) resulted in lower

Fig 5: Appropriate placement of fertilizer in 7-year old 'Arka Mridula' guava plant

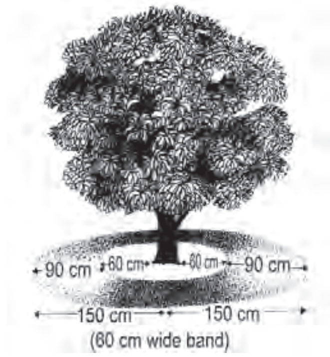

Fig 6. Appropriate placement of fertilizer in 12-year old 'Alphonso' mango

Pdff. Between scion cultivars, Italian lemon derived more P from the fertilizer than died Seedless lime (Iyengar and Keshava Murthy, 1988b). In grape, there was faster and greater uptake of fertilizer during vegetative phase than during reproductive phase. Fertilizer should be applied in two splits but within 15 days after each pruning in a band of 60cm width, between a radial distance of 60cm to 120cm (Keshava Murthy and Iyengar, 1992). Similar observations were made in 'Perlette' and 'Anab-E-Shahi' grape (Madhava Rao *et al*, 1971). In seven-year old guava 'Arka Mridula', to achieve high use efficiency, P fertilizer may be applied between 100 and 220cm during July (Fig. 5) (Keshava Murthy and Kotur, 1998b) and, in 12-year old mango 'Alphonso', the same may be applied at 60cm to 150cm radial distance during July and November, for better absorption (Fig. 6) (Keshava Murthy and Kotur, 1999a). In 'Robusta' banana, recommended P dose should be applied in two equal splits at planting, 8^{th} leaf stage (45-50 days after planting) and at 16^{th} leaf stage (90 days after planting).

Fig 7. Optimum placement of fertilizer during early vegetative (50-100 days after planting, 8-16 leaf stage) in banana

Fig. 8. Optimum placement of fertilizer after late vegetative stage (beyond 100 days after planting) in banana

The recommended N and K should be applied in four splits at 8^{th}, 16^{th} and 25^{th} leaf stages and at shooting. During early stages (8^{th} and 16^{th} leaf stage) fertilizer may be applied in 25cm circular bands between 25 and 50cm radial distances (Fig 7) whereas, during the later stages, in wider bands of 50cm width between 25 and 75cm radial distances (Fig 8). (Keshava Murthy and Kotur, 1998a).

Investigations on determining inter-plant root competition and rationalization of fertilizer management in 'Robusta' banana under high-density planting was studied (Kotur *et al*, 2011). Inter-plant competition of roots for nutrients was practically negligible. Application of 80% recommended phosphorus dose, applied in two splits as circular bands at 5cm depth, between 15 and 35cm in the first of split dose, and between 35 and 55cm distance from the base of the plant in the second split dose was optimum to attain high fruit- and bunch. In 'Surya' papaya, it was found best to apply P fertilizer between 10cm and 40cm radial distance during pre-bearing, early vegetative stage, and between 20cm and 80cm during flowering and fruit-bearing (Kotur and Keshava Murthy, 2003a; Fig. 9).

Fig 9. Appropriate placement of fertilizer in 8-year old papaya plants

Overall, application of fertilizer during rainy season and placement within the drip-circle always resulted in higher absorption of the applied fertilizer. By appropriate placement, P use efficiency of applied fertilizer increased from 0.51% to 16.35% in 'Arka Mridula' guava and 2.9% to 17.7% in 'Robusta' banana. Fertilizer use efficiency of N applied in respect of 'Robusta' banana increased from 6.8% to 60.0%. Table 4 illustrates dividends from improved fertilizer use efficiency by proper timing and placement, based on these studies. These results are valid for practical management of other fertilizers too, since, nutrient absorption presupposes prevalence of high root activity in the zone of placement at the time of fertilizer application.

Fertilizer management in vegetable crops

Area under vegetables is steadily increasing in peri-urban areas due to urbanization Concerted research carried out using ^{15}N-enriched and ^{32}P-labelled fertilizers has led to many important findings. Studies were carried out on red sandy-loam soil (Typic Haplustalf) belonging to *Thymagondlu* series, with pH 5.7, organic carbon 0.5%, cation exchange capacity 8.7 cmol (p+)/kg, available (alkaline permanganate) N 202kg/ha and available (Bray-1) P 2kg/ha.

Nitrogen

Differential behavior of vegetable crops with respect to N use

The uniqueness of each vegetable in respect of its requirement, differential dependence on soil *vs.* fertilizer source, etc. was studied in various vegetable crops. The crops differed substantially in respect of fertilizer N uptake and utilization owing to varying crop duration and specific crop characteristics. Iyengar and Shivananda (1992) observed that among the vegetables studied total N uptake was highest in brinjal, while it was the least in onion. Tomato and onion relied more on fertilizer N whereas brinjal, okra, French bean and chilli absorbed greater N from the soil source (Table 2). Brinjal had the highest average rate of N uptake (3.53/ha/day) and chilli, the lowest (0.6kg/ha/day). Recovery of fertilizer N ranged from 13.9% in chilli, to 44.7% in brinjal. Onion showed the highest physiological efficiency, while brinjal registered highest agronomic efficiency.

Table 1. Scope for improvement of fertilizer use efficiency by appropriate time and placement strategies in some fruit crops

Crop (variety)	Age (years)	Range of fertilizer use efficiency observed	
		Broadcast placement	Appropriately placed
Nitrogen derived from fertilizer (Ndff, %)			
Banana (Robusta)	1	6.8	60.00
Phosphorus derived from fertilizer (Pdff, %)			
Sweet orange (Mosambi)	9	0.51	2.36
Mandarin (Coorg mandarin)	9	1.08	4.51
Grape (Thompson Seedless)	10	2.35	3.21
Mango (Alphonso)	9	1.32	4.85
Guava (Arka Mridula)	7	2.90	17.70
Papaya (Coorg Honey Dew)	6*	0.72	6.72
Papaya (Surya)	6*	0.83	10.27
Papaya (Surya)	1	5.63	12.96

*Age in months

Cropping sequence and combination

Cropping sequences are a feature of intensive cultivation of vegetables. Here, it is necessary to identify-fertilizer efficient cropping sequence(s). Of the six cropping sequences popular among farmers of Bangalore region, it was found that French bean-tomato-onion and okra-cabbage-brinjal sequences were superior in utilizing applied N (Table 3) (Kotur and Keshava Murthy, 1999). The former was more efficient in using applied N to the first crop, than the latter. Tomato-onion-French bean sequence was the best (42.16% N utilization) due to better use of N applied to the first crop (tomato being the best user, at 30.31%) and residual N by onion (11.14%). In this manner, highly efficient cropping sequences need to be adopted in different situations to attain high utilization of applied fertilizer.

Crop combinations are also a feature essential to increase cropping intensity. Studies on the pattern of N use by component crops (Table 4) (Kotur *et al*, 2010a) showed that N use efficiency of crop combination: capsicum (onion) - watermelon (radish) – okra (French bean) was drastically reduced to 6.44-19.21% from 10.85-37.16%, compared to any of the solo (main) crops.

Management of N fertilizer use

There is a need for and scope to refine fertilizer management by adopting a strategy to ensure supply of N when a crop needs it the most, in adequate number of splits and at a place close to root zone where the crop can absorb it most efficiently. Such management enhances utilization of the applied fertilizer and, in some instances, can lead to saving of fertilizer input.

In transplanted vegetable crops, it is an accepted practice to apply N fertilizer in two equal splits: the first as basal dose, and the second as top-dressing, 20-40 days after transplanting. Basal application is likely to remain unused and may be lost to irrigation, as, the transplant is yet to develop new roots and begin absorbing applied N.

Table 2. Uptake of N, and N-use efficiency in vegetable crops in Alfisol soil

Crop	Variety	N uptake (kg/ha)		Recovery of applied N (%)	PE*	AE**
		Fertilizer	Soil			
French bean	Arka Komal	22.3	70.5	27.9	150	175
Okra	Pusa Savani	46.7	171.6	38.9	220	401
Tomato	Arka Vikas	42.7	56.8	26.4	513	340
Brinjal	Arka Shirish	53.4	401.8	44.7	223	844
Onion	Arka Kalyan	46.1	37.1	25.4	626	289
Chilli	Arka Lohit	16.7	81.8	13.9	166	137

*Physiological efficiency; **Agronomic efficiency

Fig 10. Application of N @ 90kg /ha in three equal splits delayed by 10 days after planting (bottom) saved 25% N input compared to application of full dose (120kg N/ha) as basal dose (top) in 'Arka Vikas' tomato, under Bangalore conditions

Therefore, Shivananda *et al* (1996) delayed basal N application by 10 days after transplantation, increased the number of split applications from two to three, and realized high yields of 'Arka Vikas' tomato by applying 75% of recommended N dose (Fig. 10). However, it was necessary to complete both top-dressings before 30 days from transplantation, to attain high N uptake and fertilizer utilization. In 'ECL' hybrid chilli, on the other hand, application of three equal splits, i.e., Basal + two top-dressings at 100% recommended dose, was the best provided the basal dose was applied at transplantation (owing to its quick growth). Delay in application of basal dose of N caused reduction in yield in green chilli. In 'Arka Lohit', an improved variety of chilli, deferred application in three equal splits was the best (due to the plant's slow growth habit) but no saving in N dose was possible (Kotur *et al*, 2010b). It is noteworthy that when the number of splits increased from two to three, the relative proportion of N in the first split application was reduced from 50% to 33% with the crop still young, which improved the use-efficiency of applied N.

Table 3. N utilization (%) in vegetable cropping sequences

Treated crop	Second crop	Third crop	Total
	French bean-tomato-onion		
22.20	4.48	1.57	28.25
30.31	11.14	0.71	42.16
14.19	6.72	0.54	21.45
	Okra-cabbage-brinjal		
9.46	2.03	1.74	13.30
24.47	2.48	0.56	27.51
20.20	0.62	1.91	22.73

Table 4. N and P fertilizer utilization and yield under various solo crops and their combinations

Treatment	Capsicum (onion)	Water melon (radish)	Okra (French bean)
Yield* (kg/1.8m^2)			
Solo crop-1	6.253	15.539	5.644
Solo crop-2	2.683	8.919	2.425
Crop combination	4.371	15.583	5.454
SEm (±)	0.1019	0.2537	0.1380
CD *(P=0.05)*	0.3526	0.8778	0.4774
Fertilizer N utilization (%)			
Solo crop-1	10.85	23.70	22.76
Solo crop-2	25.25	37.16	23.10
Crop combination	6.60	19.12	6.44
SEm (±)	0.590	0.843	0.821
CD *(P=0.05)*	2.042	2.916	2.839
Fertilizer P utilization (%)			
Solo crop-1	11.90	4.89	8.24
Solo crop-2	7.09	10.43	10.14
Crop combination	6.18	6.13	9.31
SEm (±)	0.146	0.128	0.229
CD *(P=0.05)*	0.504	0.442	0.792

*Yield expressed as capsicum-equivalent in capsicum (onion), as water melon-equivalent in water melon (radish), and as okra-equivalent in okra (French bean) crop combinations based on prevalent wholesale market prices

Vegetable crops are special in that they cannot complete their physiological life-cycle in the production process. Most vegetable crops are terminated much ahead of their senescence, when economic harvest has peaked. In contrast to seed-to-seed field crops, this requires high levels of nutrients to be present in the soil solution in the root zone of vegetable crops throughout the crop duration. Therefore, when the vegetable crop cycle ends, sizeable residual fertilizer nutrients inevitably remain in the soil. However, hardly 5-10% of N is utilized by the crop succeeding the first crop, and much less (<1%) in the third crop (Kotur *et al*, 2010a).

Ureolytic enzymes are closely associated with organic matter content in acidic soils, irrespective of agro-climatic region (Ramesh and Kotur, 2006; Shilpashree and Kotur, 2009). Volatilization loss of urea applied to the surface of moist soil can be substantial even in acid soils. In such instances, N use efficiency of a fertilizer can be greatly reduced unless N fertilizer is adequately incorporated into the soil or, slow release forms (or neem oil/product blended/ coated urea) are used. By such intervention, in tomato and French bean crops, neem-oil coated urea (NOCU) applied at 80% of recommended dose produced fruit/pod yield close to that obtained with 100% recommended dose (applied as prilled urea) due to its controlled release in soil, and better utilization by the crop (Kotur *et al,* 2007).

Phosphorus

Banding at appropriate depth

Phosphorus is a relatively immobile nutrient in soil. When it interacts with soil (when thoroughly incorporated/ mixed), it tends to get 'fixed' into less soluble forms, with Al^{3+} and Fe^{3+} prevalent in acid soils, or, with Ca^{2+} and/or Mg^{2+} predominance in alkaline soils. To avoid this, phosphatic fertilizers should be banded at an appropriate depth in close proximity to a zone of high root density, to attain higher P utilization (Table 3). Accordingly, banding of superphosphate at 5cm depth in French bean (Iyengar and Shivananda, 1988), brinjal and tomato (Shivananda and Iyangar, 1993), cabbage (Kotur *et al*, 1995a) and onion and chilli (Kotur *et al*, 1995b) was found to be superior. A saving of 40% in brinjal, and 20% in tomato and onion, was possible without any loss in yield. In deep-rooted okra crop, however, deeper placement (10cm depth) was necessary to attain this (Shivananda and Iyangar, 1990).

In the case of F1 hybrid varieties which, in most cases, are more vigorous than improved or high-yielding cultivars, roots are likely to grow deeper. Therefore, in hybrid capsicum, a crop grown from pro-tray raised seedlings (Kotur, 2008), and in crops of cabbage and cauliflower raised from raised bed seedlings, superphosphate needed to be banded at a deeper depth of 10cm to attain high yield and optimum fertilizer use efficiency, as, the roots grew much deeper in these cases. However, in F1 hybrid 'Arka Ananya' tomato grown in raised bed seedlings, a shallower banding at 5cm depth was found to be superior to deeper placement, and led to a saving of 20% P and, yet, achieved high yield. This was attributed to the fact that tomato produced a tuft of secondary roots at around 5cm depth which absorbed most of the applied fertilizer P (even though the tap root, perhaps for proceeding anchorage, extended up to 12cm depth). In all these crops, attempts to elicit root activity distribution in a crop grown by transplanting raised-bed/ pro-tray seedlings using soil injection of ^{32}P, did not succeed. Perhaps this was because plant part (shoot/ leaf) was not sampled accurately or detection of ^{32}P was too sensitive.

Phosphorus use efficiency under increased cropping intensity

Under enhanced cropping intensity in 'capsicum (onion)-water melon (radish)-okra (French bean)' crop combination (crops mentioned in parentheses here are intercrops), P use efficiency drastically reduced from 4.89-11.90 to 6.18-9.31%, compared to either of the sole (main) crops (Kotur *et al,* 2010a). Reduction in P utilization under various crop combinations was smaller than that in N utilization (Table 4).

It is evident that fertilizer use efficiency can be substantially improved (Tables 1 and 6) by appropriate fertilizer management, through simple and non-monetary innovative practices. Considering that >30% of nitrogen and entire quantities of phosphoric and potassic fertilizers are imported by India, improvement by even 1% use efficiency of N, P and K means saving several hundred crores of rupees, besides making farming enterprise more cost-efficient and eco-friendly.

Identification of nutrient-efficient varieties of scion and rootstocks in fruit crops

Inter-specific and inta-varietal differences in kinetic parameters (I_{max}, K_m and C_{min}) leading to different rates of absorption, and, their response to variations in rhizospheric nutrient concentration or marginal soil situations in respect of P, were studied in important fruit crops using ^{32}P as a tracer. Rate of net inflow (I_n) was calculated by the formula:

$$I_n = \frac{I_{max}\ (C_s - C_{min})}{K_m + (C_s - C_{min})}$$

Where, I_{max} denotes maximum rate of nutrient absorption (in pmoles/cm/s); K_m, the nutrient concentration at which inflow is $\frac{1}{2}I_{max}$ (in pmoles/l); C_{min}, the nutrient concentration at which there is no net inflow (in pmoles/l); and C_s, the equilibrium concentration of P (at 10µmole/l) in the solution. Although such studies were widely conducted on annuals and short-duration plants, it is worthwhile to study the phenomenon in perennial fruit trees (which conserve nutrients for future use, to sustain a cyclic growth pattern).

Table 5. Comparison of different levels of phosphorus banded at 5cm below seed/plant row

Level of (kg/ha) P_2O_5	Recommended (%) P dose	Uptake (kg/ha)	Fertilizer P Yield (t/ha)	Fertilizer P Utilization (%)
Tomato				
60	80	23.81b	5.74	21.90b*
75	100	19.50a	5.33	16.27a
Brinjal				
24	60	6.41	2.61	24.89b
40	10	4.10	2.44	13.94a
Onion				
40	80	63.43	4.79	22.95
60	100	65.65	4.86	18.63
Chilli				
48	60	13.09	5.54	26.54
80	100	17.98	7.17	20.61
Cabbage (Pride of India)				
48	60	9.01	8.97	40.11
80	100	9.34	12.27	35.26
Cabbage (Mahyco Hybrid 413)				
72	60	14.20	14.45	46.15b
120	120	14.35	17.52	33.59a

*Number followed by the same alphabet indicates no statistical difference *(p=0.05)* in each crop

Table 6. Scope for improvement of fertilizer use efficiency by application at appropriate time and placement in some vegetable crops

Crop (variety)	Fertilizer use efficiency observed Lowest	Fertilizer use efficiency observed Highest
Nitrogen		
Tomato (Arka Vikas)	22.4	27.9
Chilli pepper (Arka Lohit)	16.9	30.3
Chilli pepper (Hybrid ECL)	11.0	22.1
Phosphorus		
French Bean (Arka Komal)	12.4	19.2
Okra (Pusa Savani)*	7.6	12.6
Onion (Arka Kalyan)	18.6	23.0
Chilli pepper (Arka Lohit)	15.4	20.3
Cabbage (Pride of India)	31.5	35.5

Amongst polyembryonic mango rootstocks, 'Peach' and 'Prior' may be said to be highly responsive to applied P, and likewise, scions budded onto them, in view of higher values of I_{max} observed. 'Nendran' banana, 'Bangalore Blue' grape and 'Citrumelo' citrus could also be fertilizer responsive. Low values of C_{min} observed in 'Rough Lemon' rootstock of citrus, 'Vellaikolumban' polyembryonic rootstock of mango, and 'Anab-e-Shahi' variety of grape indicate that these rootstocks/varieties of crops can be grown successfully in marginal soils. On the other hand, most citrus

Table 7. Phosphorus derived from fertilizer (Pdff, %) in newly-developing and mature old parts in Italian lemon as influenced by various rootstocks

Rootstocks	Young, developing parts: Shoot tip	Young, developing parts: Flower	Young, developing parts: Fruit	Mature leaf	Mature fruit: Rind	Mature fruit: Juice
Rough lemon	5.9	8.8	2.5	4.5	2.2	1.2
Rangpur lime	5.3	7.2	4.7	5.5	4.8	3.2
Cleopatra mandarin	5.4	5.7	6.2	4.7	2.2	2.8
Kodaikithuli mandarin	10.2	12.6	5.1	8.4	1.5	3.2
Trifoliate orange	8.1	10.5	6.1	7.2	2.8	2.8
Carrizo citrange	11.7	16.2	13.0	10.2	3.2	5.7
Citrumelo	12.5	16.7	7.5	10.5	4.6	2.3

Table 8. Appropriate quantities of urea and sulphate of potash, cost per banana bunch and expected increase in bunch weight in different varieties of banana

Banana variety	Quantity of urea and sulphate of potash used/bunch (g)	Cost/ bunch (Rs.)	Improvement observed: Weight of the bunch (range, in kg)	Improvement observed: Pulp:Peel ratio (From/To*)
Grande Naine	10	3	3-5	2.70 to 2.84
Dwarf Cavendish	7.5	2	2-4	2.84 to 3.74
Robusta	7.5	2	2-4	2.57 to 3.22
Nendran	7.5	2	2-4	3.28 to 4.08
Ney Poovan	2.5	1	1-3	4.44 to 6.09
Najangud Rasabale	5	2	1-3	3.11 to 4.60
Red banana	7.5	2	2-4	4.40 to 5.45

*Pulp:Peel ratio in fruit from 'Control' to 'Treated' bunches

Table 9. Activity of tritium (dps g^{-1} dry weight) in mesocarp and seed tissues in 'Alphonso' mango fruit treated with PGRs

Fruit status	Mesocarp	Seed
Healthy	506	39
Spongy	1467	68
Spongy (germinating seed)	3185	123
Spongy (GA_3-treated)	3180	189
Healthy (PBZ-treated)	495	34
CD (*p=0.05*)	17.9	

(rootstocks/scion) varieties require more fertile soils. Phosphorus inflow rate (I_n) is comparatively high in polyembryonic mango rootstocks particularly, 'Olour', 'Peach', 'Prior' and 'Vellaikolumban', and thus, better P absorption efficiency, and may respond better to application. Among banana varieties, also known to have high inflow rates, 'Nendran' responded better to fertilization compared to others. Similarly, 'Bangalore Blue' grape may likely respond better to P fertilization. All citrus rootstocks, which incidentally showed lowest P inflow rates, may perhaps show poor response to P fertilization (Keshava Murthy and Kotur, 2000).

In terms of suitability of soil to a variety, or response of a variety to applied P, fruit crops and their varieties differed widely. These differences may be partly due to kinetic parameters of absorption, or, due to root morphology or ontogeny of the crop/variety. An ideal situation for a rootstock or variety would be to have higher I_{max} and lower K_m, which makes it both fertilizer responsive suitable for marginal soils. However, verification trials in the field are required to confirm influence of these kinetic parameters of P absorption for future selection or breeding work.

Other applications

Need-based, ingenious applications of tracer techniques can be made to address specific experimental requirements. Some instances are mentioned below.

Mobilization of P from mother plant to sucker in 'Robusta' banana

Balakrishnana and Shanmugavelu (1985) showed that during early stages of growth, suckers caused considerable nutrient depletion to the mother plant by drawing large proportion of their requirement from it. Rajeevan *et al,* (1987), on the other hand, showed that considerable mobilization of P from mother plant to the sucker was possible by retaining the pseudostem for some time after bunch harvest. Keshava Murthy and Iyengar (1991) found that in 'Robusta' banana, retention of pseudostem of the mother plant even upto even 45 days after harvest of bunch was beneficial.

Contribution of exogenous fertilizer source to phosphorus requirement in citrus

In fruit plants, understanding the role of energy reserves and mineral nutrients in the permanent framework of a tree, for growth and yield, is important to rationalize fertilizer management. In newly developing and mature parts of *Citrus limonia* (Italian lemon) raised on various rootstocks, new growth obtained a maximum of 15-16% of its phosphphrus content from exogenous fertilizer source. Assuming that at least equal proportion came from the soil source, endogenous source of reserve phosphorus within the plant contributes at least 65-70% of phosphorus requirement for current growth of the newly-developing parts (Iyengar and Keshava Murthy, 1988b; Table 7). Similar results were obtained in Seedless lime. It was further demonstrated that in declining 'Thompson Seedless' grapevines, the entire energy (carbohydrates, that largely constitute dry matter) and N, P and K requirement of current vine parts was met by reserves present in the permanent vine parts (Keshava Murthy and Kotur, 2001).

Internal dilution technique for comparing sources not amenable to isotope labelling

In banana, carrier-free ^{32}P was injected into the rhizome to compare superphosphate (SSP), farm yard manure (FYM), tank silt (TS) and combinations thereof as a source of phosphorus. Among these, SSP showed the lowest PdES (phosphorus derived from external source), while, TS showed higher values, with FYM+TS being the best. FYM treatments, with or without SSP, showed up as intermediate. Tank silt (TS) had higher P availability in soil, followed by FYM (Keshava Murthy and Kotur, 1999b). Thus, it was possible to evaluate various sources of P without having to label the sources.

Determining tree volume infruit trees using isotope dilution technique

Similar to determining volume of water in large water bodies, using the principle of isotope dilution ^{32}P was injected into a tree trunk and allowed complete distribution. Thereafter, its dilution was estimated as a measure of tree volume. Thus, volume of 4-year old 'Alphonso' trees (56.903-68.981 $m^3 \times 10^3$) and their biomass (321.70-36.23g $\times 10^3$) showed good agreement but, generally, over-estimated both by about 19% compared to actual values determined physically (43.720-60.693 and 22.88-37.78, respectively; Kotur and Keshava Murthy, 2004b). The technique was applied to estimate tree volume of 15-year old 'Alphonso' mango and 12-year old 'Allahabad Safeda' guava trees (Kotur, 2004). This holds promise in stusies on fertilizer use efficiency and nutrient dynamics of tree crops where total biomass of the tree is an important trait.

Detection of unorthodox movement of nutrients in banana

A technology for enhancing size of fingers in a banana bunch was developed to suit market demands, by de-navelling and post-shooting feeding of N, K and S through the distal stalk-end of rachis (Kotur and Keshava Murthy, 2008). The technique involves blending approximately 7.5g urea and 7.5g sulphate of potash dissolved in 100 ml water with 500g fresh cow dung, and applying the slurry to de-navelled stalk-end of 'Robusta' bunch soon after fruit-set. About 10-15cm long rachis should be available after the last banana hand to tie a plastic bag (used milk bag is convenient) with a strong string (Plate 1). Experimentally, it was found that by this treatment, bunch weight increased by 67% over the 'control' where the male flower was retained until harvest. Using ^{15}N-isotope label, movement of 51% of N into the bunch from ammonium sulphate blended in cow dung was confirmed. In 'Ney Poovan' banana, blending 5g ammonium sulphate (or 2.5g urea) and 2.5g sulphate of potash and applied similarly, showed a response of 78% by absorbing 42% of the N applied (Kotur and Keshava Murthy, 2010)(Plate 2). Quality of banana in terms of pulp:peel ratio distinctly improved by direct feeding of nutrients to the bunch. The technology was standardized in seven popular cultivars of banana (Table 8) and was very cost-effective.

Plate 1

Plate 2

Nitrogen, phosphorus and sulphur enriched cow dung applied to de-navelled distal end of 'Robusta' banana bunch (top) and response observed in 'Ney Poovan' banana (bottom; Left: Control, Right: Treated).

Monitoring internal dynamics of water in mango fruit

Using ^{3}H tritiated water, internal movement of water was successfully monitored in investigations on mitigating spongy tissue in 'Alphonso' mango (Ravindra *et al*, 2010). An up-regulation of seed metabolic activity with GA_3 accompanied by significant increase in tritium counts, and down-regulation of seed metabolism with PBZ resulted in a decline of tritium counts in seed (Table 9) thus establishing a direct relationship between mobilization of water from the mesocarp to the embryo and metabolic activity of the seed.

Source-sink relation studies in horticultural crops

Mango: Studies on translocation and *in planta* distribution pattern were undertaken using compounds labelled with ^{14}C-isotope during different phases of growth and development in mango (Kohli, 1985). Mango cultivars on which these studies were carried out were: 'Alphonso', '*Dashehari*', '*Kalpady*' '*Langra*', 'Kensignton', '*Neelum*' and '*Bangalora*'. (i) Newly emerging growth flush in mango received large quantities of assimilates produced elsewhere. Expanding leaves in the new flush continued to import photosynthates from older leaves until turning dark green (4-week old); (ii) In 6-month old epicotyl grafts, photosynthates moved largely to the root system and to new growth flushes in the vegetative phase. Presence of growing fruits substantially enhanced percentage of photosynthates exported from leaves, to meet the needs of rapidly growing fruits; (iii) Young inflorescence acted as a strong sink and, at later stages, inflorescence-leaves photosynthesized and contributed to development of fruits; (iv) At pea stage of fruit development, export of photosynthates from ^{14}C-fed leaves increased from 50 to 89% at fully maturity. Flow of photosynthates slowed down as fruits attained maturity after a stage of rapid growth; and, (v) In comparison to fruit pulp, the seed (kernel) acted as a stronger sink.

Based on results of translocation studies using CO_2 in different varieties of mango, further studies were conducted. Feeding CO_2 resulted in higher rate of carbon fixation in leaves of girdled shoots than that in 'control' shoots; but, translocation of ^{14}C assimilates to the developing fruits on girdled and 'control' shoots was comparable (Chacko *et al*, 1982). In both biennial- and regular-bearing cultivars, there was progressive reduction in fruit size with decreasing number of supporting leaves. Biennial-bearing cultivars required over 50 leaves, whereas, regular-bearing cultivars needed around 35 leaves for normal development of the fruit. Utilization of reserve photosynthates from vegetative organs during the 'on year' could be a contributing factor for towards biennial- and erratic-bearing in mango (Reddy and Gorakh Singh, 1990, 1991; Reddy and Kurian, 1993). Paclobutrazol application seemed to alter source-sink relationship in mango, and, lesser number of leaves were able to support fruit growth (which could be a reason for enhanced fruit-yield despite suppressed vegetative growth) (Kurian *et al*, 2001).

Vegetable crops

Dynamics of photosynthates in vegetable crops

Salient findings using ^{14}C-isotope are: (i) In eggplant, transport of assimilates was bi-directional within 48 hours, ^{14}C moved to all parts of the plant: root, stem and leaves, until fruits began to develop. Fruits subtending the fed leaves were a major sink (Srinivasa Rao, 1988); (ii) Genotypic differences in partitioning of carbon in different plant parts were observed; these may play a major role in determining content of solids in tomato cultivars (Srinivasa Rao and Bhatt, 1989a); (iii) In okra, translocation of assimilates was bi-directional to apical and basal portions of the stem. Irrespective of the leaf fed, subtending fruit was the strongest sink among reproductive parts (Srinivasa Rao and Bhatt, 1989b); (iv) In fruiting bell-pepper plants, the first flowering node acted as a major sink for carbon (10.2%) for up to 20 days, and weakened thereon as other growing fruits overtook and used up 46.7% of carbon. In de-blossomed plants, maximum amount of radiocarbon moved to roots due to slower growth of the other vegetative organs during summer (Bhatt and Srinivasa Rao, 1993a); and, (iv) In okra, pod was a strong sink (26.2%), but the stem also accumulated 34.4-42.1% of ^{14}C indicating, that, the latter could act as a storage organ for possible re-translocation later to developing fruits (Bhatt and Srinivasa Rao, 1993b).

Studies on moisture-stress tolerant and susceptible onion: Ratio $^{18}O/^{16}O$ reveals net carbon sequestration as a function of respiratory loss and soil-water deficit. Water stress was imposed in four cultivars: SM-11 (tolerant), N-2-4-1 (moderately tolerant), 'Arka Niketan' (susceptible) and 'Arka Kalyán' (tolerant) at 30 and 60DAP, for four weeks. Low $\nabla^{18}O$ indicated highly water-use-efficient cultivars that also exhibited high levels of transpiration. High correlation was observed between oxygen (^{18}O) enrichment and stomatal conductance in various onion cultivars (Fig.11).

Possibilities of using tracer techniques are immense in the multi-disciplinary mode in horticultural research. The present paper provides only a partial glimpse of the vast scenario. Requirement for special design of isotope-dedicated field and laboratories, trained staff and high cost make it a daunting task in the short run; but, its multiple utility to various disciplines, quick and reliable results when a focused programme is in place, make it a cost-effective and invaluable technique in the long run. The present article proves this in ample measure. Isotope–aided studies should address simple and specific questions ordinarily not solved by conventional research, and provide direct answers. Practical solutions useful to growers can emerge with adequate research using conventional techniques based on leads provided by isotope-aided studies.

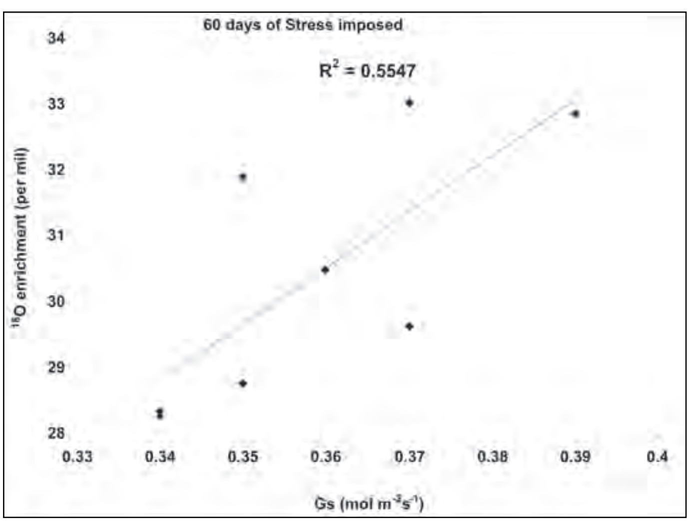

Fig 11. Correlation between oxygen (^{18}O) enrichment and stomatal conductance in onion (Srinivasa Rao, 2006-07)

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