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Understanding *Cuminum cyminum*: An important seed spice crop of arid and semi arid regions

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Abstract

Cumin (*Cuminum cyminum* L.) with chromosome number of 2n= 2x= 14 is an economically and pharmaceutically important small herb of family *Apiaceae*. It is mainly cultivated in India, Iran, Syria, Pakistan and Turkey. It is widely used in foods, beverages, liquors, medicines, toiletries and perfume industries. The mature dried fruit contains 2–5% essential oil. Though it is an important plant among seed spices, it suffers with various inherent problems. Low germination rate and poor seedling establishment are one of the major problems in cumin. Similarly, being a monotypic species, phenotypic and genetic variability in cumin is also low. Because of which there is no source of resistance available against biotic (*Alternaria* blight and *Fusarium* wilt) and abiotic (frost and salinity) stresses. The flowers being small and slender restricts possibilities of artificial pollination creating hinders in following recombination breeding for genetic improvement. Consequently, most of the varieties available today are developed by selections. A large number of efforts on identification of bioactive constituents and verification of pharmacological effects have been recorded in cumin. As compared to cumin's classical breeding including mutagenesis, very less effort have been made on molecular breeding. Therefore, practically no molecular tools are available and no genomics based breeding programmes for improvement of cumin have been initiated yet. In this communication, we underline the research attempts made in cumin on various aspects like genotype (G) and environment (E) effects on the seed biochemical constituents, mutation breeding, variability studies, tissue culture and genetic transformation, molecular markers studies and some future directions of research are also suggested.

Key words: Breeding, cuminaldehyde, genetic divergence, molecular marker.

Introduction

The genus *Cuminum* is derived from the Greek word 'Kuminon', itself probably derived from the Babylonian ka-mu-na. Cumin (*Cuminum cyminum* L. or Jeera), 2n = 14, with medicinal, pharmaceutical and nutraceutical properties is a member of subtribe Carinae of the tribe Ammineae of family Apiaceae (Drude, 38). Origin of cumin is still in dispute but it is native to Northern Africa, and has spread via West Asia to Central Asia. It is one of the old cultivated medicinal food herbs in Asia, Africa and Europe (Gohari and Soodabeh, 48). The crop is mostly grown in Morocco, Turkey, Greece, Egypt, Iran and the southern part of the Mashad province (Charles, 30). The chief cumin exporters are India, Syria, Pakistan, and Turkey while India and the USA are main oil producers.

Cumin is extensively used in foods, beverages, liquors, medicines, toiletries and perfumery, and is grown in the mild climate of Gujarat, Rajasthan, Haryana, Madhya Pradesh and Uttar Pradesh (Azeez, 12; Saxena *et al*., 97). Its seeds have been commonly used for culinary and flavouring purposes due to strong and aromatic flavour (Deepak, 36) and also in ethanomedical therapy since ancient times in various countries. Globally, India is largest producer (70%) as well as consumer of cumin (Sastry and Anandaraj, 94). Bangladesh, Brazil, Japan, Malaysia, Nepal, Singapore, the UAE, the UK, the USA are chief importer of Indian cumin.

Although a number of potential species (ca 18) have been described based on morphological differences, merely four species names (*C. borszczowii, C. cyminum, C. setifolium and C. sudanense*) are botanically acceptable (Anonymous, 8). Most studies in respect of seed structure, seed biochemistry and crop improvement have been focused on the popular agronomic species i.e. *Cuminum cyminum*. This review aims at exploring the physiological and biochemical properties of cumin seed and the impact of genotype ×

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environment (GE) interactions on the main biologically active compounds available in the seed. The review also investigates different approaches for genetic improvement for consistent improvement in cumin seed quantity and quality.

Crop description

Cumin is an annual herbaceous plant which grows up to 15-50 cm height somewhat angular and tends to drop under its own weight. The leaves with bluish-green hue, 5-10 cm pinnate or bi pinnate, are alternate, simple or compound and have a sheathing leaf base below. Flowers are on a compound umbel (arrangement of flowers looks like an umbrella) and each umbel has 5-7 umbelletes (Sastry and Anandaraj, 94). The umbels of the first order show the maximum number of umbellets and maximum of flowers in each umbellets. Due to unequal sepal size in cumin, the flowers of the outer whorl of umbellets are zygomorphic ("yoke shaped", "bilateral") while those in inner are actinomorphic ("star shaped", "radial") (Sehgal, 99). The flowers are bisexual with colours like pink and red growing on the inflorescence compound umbel up to 3-5 mm in diameter (Charles, 30). Stamens are antisepalous with long filaments and dithecous anthers. The flowers have bicarpellary, syncarpous and inferior ovary that develops into a very characteristic fruit called a cremocarp (Parashar *et al.,* 85). Ovary shows prominent ridges and furrows. In cumin, along the ridges the wall of the fertilized ovary shows 21-26 layers of cells of which 3-5 subepidermal layers are chlorophyllous. Like coriander, cumin shows multi-celled archesporium (Sehgal, 99).

Cumin is a cross-pollinated spice and honey bees help in pollination. The ethereal odour of the plant attracts insects. Cross pollination is essential due to well-marked protandry, and the anthers dehisce even before the style and stigma are fully developed. The stylopodiums secrete nectar, attracting pollinators like flies, mosquitoes, gnats, beetles, moths, and bees (Boriss, 27). In cumin, rare cause of polyembryony arises due to proliferation of suspensor archesporium (Sehgal, 99).

The seed: structure and compounds

At maturity, capsular cremocarp invariably breaks into two albuminous seeds with copious endosperm and a minute embryo; with a ribbed wall consisting number of longitudinal oil canals. The fruit dehisces by splitting into two mericarp (one-seeded part). Though these grain-like fruits are called the seeds, the true seeds are within them and come out only during germination through disintegration of the fruit wall. The fruit is a lateral fusiform or ovoid achene 4–5 mm long, containing a single seed which are thicker in the middle, compressed laterally (Gohari and Soodabeh, 48). The fruit is sometimes brownish or yellow with slightly curved schizocarp. The seeds are approximately 2–3 mm long and 2 mm thick with a light brown and a yellow hue. According to the microscope observation, three different fragments can be observed in fruit: the seed coat, ridge and endosperm. Endosperm is polygonal cells and containing microspheroidal crystals of calcium oxalate. Endocarp is large, thin-walled, elongated cell; found mostly attached with vittae and parenchymatous cells of mesocarp. The fibro-vascular tissue and testa is also present (Rai *et al.,* 89). Fruit surface has five primary ridges alternating with four less distinct secondary ridges bearing numerous small prickles hardly visible to the naked eye (Amin, 7). Between each primary ridge is an elongated oil tube (vitta) besides 2 vittae on the face of the mericarp. Mericarp has six vascular bundles and six vittae (schizogenous canal) alternate theses vascular bundles. The vittae secretes essential oil and are yellowish-brown, consisting of small, thin-walled, polygonal, tubular, cutinized cells (Rai *et al.,* 89; Kapoor and Kaul, 63). Mericarp contain about 2.5 - 5 per cent of an essential oil, which is lighter than water, pale yellow to colourless, and has the sensible properties of the fruits. The volatile oil should be kept in well-sealed bottles or aluminium containers (Peter, 86).

Seed chemical compounds

The ripe, dried fruit contains moisture 7%, fiber 17%, carbohydrates 29%, fatty oil 4%, proteins 18%, ash 6%, and essential oil 2–5% (oil has cuminic aldehyde 33%, b -pinene 13%. terpinene 25%, p-cymene 8.5%, p-mentha-1,3-dien-7-al 5.6%, b-farnesene 1.1%), flavonoid glycosides, tannin, resin, and gum (Charles, 30). In addition, the seeds yield numerous free amino acids, and a variety of flavonoid glycosides, including derivatives of apigenin and luteolin. Cumin seeds contain up to 2.5-5% of a volatile oil composed primarily of aldehydes (up to 50-60%) and hydrocarbons (30–50%) (Azeez, 12; Parashar *et al.,* 85). Cumin also contains non-volatile chemical components including tannins, oleoresin, mucilage, gum, protein compounds and malates. The oleoresins are obtained by subjecting the ground cumin to different organic solvents such as n-hexene, ethanol, methanol etc. (Peter, 86).The seed chemical constituents vary remarkably depending on their varietal and ecological factors (Rebey *et al.,* 92; Sowbhagya, 114).

The average characteristics of commercial oil are: specific gravity 0.900–0.935 (at 25°C); optical rotation

+4° to +8° (at 20°C); refractive index 1.495–1.509 (at 20°C). Total dietary fiber in cumin is not only high before oils and non-volatiles extraction but also in spent residue after oil extraction (Nadeem and Riaz, 78). Results shows that the total dietary fiber content (TDF) of cumin is 59.0%, insoluble dietary fiber (IDF) 48.5%, and soluble dietary fiber (SDF) 10.5%, while the spent residue from cumin has been found to contain 62.1% TDF, 51.7% IDF and 10.4% SDF. Cumin also contains safrole, a mutagen, which is degraded by cooking (Farag and Abozeid, 45). The spent residue also contains 7.7% starch and 5% bound fat (Sowbhagya *et al.,* 115). Thus, spent residue can be used in as animal feed for cattle or as broiler feed and as a manure source. Saxena *et al*., (98) attempted extraction of essential oil from cumin crop residue and succeeded in recovery of significant quantity of essential oil. GC-MS profiling of extracted oil revealed presence of all important constituents though in slightly less in quantity as compared to the oil extracted from cumin seeds.

Cumin essential oil mainly contains monoterpene aldehydes while sesquiterpenes are minor constituents of cumin oil. Cuminaldehyde (4-isopropylbenzaldehyde) is the main active compound of cumin oil along with limonene, eugenol, α- and β-pinenes and some other minor constituents have been found in cumin oil (Gohari and Soodabeh, 48). It is responsible for aroma and also for the bitterness in cumin (Hirasa and Takemasa, 53). In non-heated whole seeds, 3-p-mentha-7-al and cuminaldehyde in combination with other related aldehydes are responsible for characteristic aroma. However, it is reported that naturally occurring aldehyde in fresh cumin is 1,4-p-methadien-7-al and cuminaldehyde while the other related compounds are only artifacts formed either during storage of ground seeds or distillation of oil (Borges and Pino, 26). As per Singh *et al*., (108) cuminaldehyde is likely to impart good corrosion inhibition activity due to presence of aromatic ring, aldehyde and isopropyl groups as substituents. They also reported that cuminaldehyde has an excellent inhibition effect for the corrosion of mild steel in 1 M HCl. The high inhibition efficiencies of cumin seed extract were attributed to the adherent adsorption of the inhibitor molecules on the mild steel surface. UV-Visible spectroscopy also confirmed a complex formation between the inhibitor and the mild steel surface.

In cumin, much of the literature has been published about its oil, aldehyde, alcohol contents, analysis of its essence and physical properties. The residue remaining after volatile and fixed oils extraction is rich in protein and carbohydrates and can be used as a cattle feed or a source of protein concentrate (Badr and Georgiev, 17). However, the nitrogen content, free amino acids and amino acid compositions of its protein have been studied occasionally. Toghrol and Daneshpelouh (116) have reported the amino acid composition, free amino acid, total nitrogen and protein nitrogen content of Iranian cumin seeds. Though, the level of free amino acids in cumin seeds is not high, it contains 14 free amino acids, five of which are essential amino acids. Similarly, crude protein, true protein, non-protein nitrogen and amino acid composition were determined in cumin seeds at Bulgaria for two seasons (Badr and Georgiev, 17). Protein content in this study ranged from 18-23% with 18 amino acids of which eight were essential amino acids. The difference in number of amino acids and essential amino acids between studies could be due to the methods applied for the protein hydrolysis or the method used for amino acid determination. However, in both studies, glutamic acid was found in highest amount. Badr and Georgiev (17) eventually, concluded that the cumin protein is of a low biological value. It is of less importance as a protein source alone and could be useful only if mixed with other sources of protein that supply adequate amounts of methionine, cystine, phenylalanine and tyrosine, since the cumin protein is virtually devoid of these important amino acids.

The cumin seeds contain 14.5% total lipids on dry weight basis. Silica column based lipid fractionation showed that total lipids consists 84.8% neutral lipid, 10% glycolipids and 5.1% phospholipids. With both saturated and unsaturated fatty acids, oleic, petroselenic, and linoleic acids accounts for 70% of the total fatty acids in cumin (Sowbhagya, 114). The level of petroselinic acid detected in study of Rebey *et al.,* (92) was similar with the findings of 51.27 % by Shahnaz *et al*., (100) but lower than the report of 83.4 % by Hemavathy and Prabhaker (52). This difference could be mainly due to the effect of genetic factors linked to the variety and its interaction with environmental factors, edaphic conditions and cultural practices followed. A genotype that generates a very high level of a phytochemical at a given location may not produce the same level of that specific phytochemical in another (Zandi *et al.,* 120).

Recently, Zaman and Abbasi (119) has characterized of a nonspecific mono-meric lipid transfer protein nsLTP1 (7.9 kDa), involved in transport of lipids between membranes from cumin seeds by mass spectrometry, circular dichroism (CD) spectroscopy and amino acid sequencing. The three-dimensional structure of cumin nsLTP1 liganded with myristic acid and Lyso-myristoyl

phasphatidyl-choline has been generated using homology modeling to gain insight into its functioning. They also concluded that nsLTP1 may perform an important physiological role in constitutive plant defence mechanisms, working in an orchestrated manner with a vast group of secreted defensive compounds such as vicilins, hydrolases and cystatins.

Cumin oleoresin is a solvent extracted product from ground or crushed seed, extracted product is solvent free brown to yellow green resin containing 30–50% of volatile oil, and other non volatile and volatile flavour components. Recently, Sharma *et al.* (101) studied the effect of cryogenic grinding on oleoresin properties of seed extract of two cumin genotypes and found that cryogenic grinding not only retains the volatiles in seeds of popular varieties RZ-209 and GC-4 but enhanced the recovery also. A significant increase (28.28%) in oleoresin percentage was observed when RZ 209 seeds were milled using cryogenic grinding. This increase was, however less (16.046%) in cv. GC4. Cumin oleoresin finds application in sauces, crackers, meat and sausages. Cumin oleoresin is available in market and can replace the original cumin seed powder with a standardized taste and aroma that can be tailored as per the requirement of the product. Oleoresins have longer shelf life, are cleaner (no bacterial contamination) and are a convenient substitute for ground spices.

Effect of water stress on yield and quality of cumin

Cumin, predominately being a crop of arid and semi arid area faces water stress problems in general. Limited water supply significantly affects growth and metabolic activities and may also causes negative effect on plant growth and development (Rebey *et al.,* 92). Interestingly studies conducted by Jangir and Singh (58), Yava and Dahama (118) and Ahmadian *et al*., (3) recommended five, four and three irrigations per season for cumin production under different conditions, respectively. A comparative study of two irrigation patterns *i.e.*, only one irrigation at the time of sowing and two irrigations (at sowing and seed formation) showed that under two irrigations cumin produced higher seed and essential oil yields, but essential oil content decreased with increasing irrigation number (Nejad, 80). Motamedi-Mirhosseini *et al*., (77) showed positive association of irrigation and yield as cumin accessions studied under three and five irrigations responded for higher yield and the most important yield components under five irrigation regimes. There is limited information available regarding the impact of abiotic constraints on bioactive compounds of cumin, though Bettaieb *et al*., (25) found significant effect of water constraint on biochemical composition of cumin aerial parts.

An interesting study done by Rebey *et al*. (92) showed response of cumin under severe water deficiency (SWD), a 25 % reduction in water supply than control significantly reduced number of umbels and the number of umbellets per umbel, concomitant with the decrease in 1000 seed weight and seed yield. Under SWD the variance for seed yield varied from 3.03 to 6.62 g. These findings suggested that cumin is more adapted to moderate dryness and is not much sensitive to drought such as caraway (Laribi *et al.,* 69) and other aromatic crops like *Ocimum basilicum*. Bettaieb *et al*., (25) reported that higher cumin seed yield under moderate water stress and reduced levels under severe drought conditions.

Response of cumin to both irrigation water and micronutrient were studied by Mazaheri *et al*., (75), three irrigation treatments (including control and stopping irrigation at vegetative and reproductive stages) were taken as main plot and four levels of micronutrients (including control, Zn, Mn and Zn+Mn) as sub plot. They found that stopping irrigation at vegetative stage led to decrease of seed yield and biomass to about 28.2 and 21.6% against control treatment. Essential oil yield of cumin decreased significantly with water stress while micronutrients showed no effect on yield of essential oil. Likewise, Rebey *et al*., (92) reported 37.19% decreased in oil yield under SWD than control conditions, but under moderate water deficiency (MWD), yield was 1.40 times higher in comparison to the control. Thus, only MWD enhanced cumin essential oil yield. Proportion of cuminaldehyde increased up to 23.53% under MWD. Besides, total phenolic contents were higher in the treated seeds (MWD and SWD). They also explained that increment in essential oil production under a moderate drought level could be due to the reallocation of the photosynthates for the biosynthesis of stress protecting metabolites as defence biochemical adaptation mechanism.

On the other hand, study of Alinian and Razmjoo (5) revealed that drought stress had no marked effect on plant height, number of branches per plant and number of umbelletes per umbel of four accessions. Aerial essential oil content increased under moderate, but was not affected by severe drought stresses indicating that less affect of genotypes. However, number of umbels per plant, number of seeds per umbel and 1000 seed weight were reduced as drought level increased. Comparison between two stress and non-stress conditions showed that number of umbels per plant, number of seeds per umbel and 1000 seed weight

reduced under drought (Motamedi-Mirhosseini *et al.,* 77). Reduction in biological and seed yield of cumin was also reported by Ahmadian *et al*., (3). Reasons for contrasting results between various reports could be due to differences between drought severity and duration, the metabolic state of the plant, plant species, plant developmental stage and even cultivars of the same species (Bettaieb *et al.,* 25). Rahimian (88) concluded that in Mashhad region of Iran, maximum yield can be gained by complete irrigation diet. While, in a three-year study in same region (i.e. Mashhad) Sadeghi and Mohasel (93) observed the reverse phenomenon; in ordinary considering precipitation (250 mm a year), the effect of irrigation on yield enhancement was not significant and on the other hand crop growth was also suppressed.

Results of Rebey *et al.,* (92) showed that oil yield (%) of cumin seeds decreases significantly by about 25.55 and 48.39% under MWD and SWD, respectively, in comparison with the control. They were first to investigate the effect of drought on fatty acid composition in cumin seeds. They found that MUFA decreased by 22.34 and 20.15%, respectively, under MWD and SWD; and this decrement was mainly mediated by the reduction of petroselinic acid proportion. However, drought affected the PUFA fraction, which decreased by 20.14 and 47.81%. In Contrast to UFA, drought stress increased SFA fraction as well as the proportion of the major SFA, palmitic acid, whose proportion raised by 1.43 and 1.64 folds, in comparison with the control under MWD and SWD, correspondingly.

Seed priming

Low germination percentage in seeds of *Apiaceae* family has been already reported (Khosh-khui and Bonyanpour, 66) including cumin where high variation from 8.4 to 80.5% in germination was also observed (Arslan and Bozkurt, 9). In comparison to India, globally cumin is mostly cultivated in rain-fed system, Therefore, seed germination and poor seedling establishment is a major problem in cumin cultivation. Rapid seed germination and stand establishment are critical factors for crop production under stress conditions. Cumin requires 10 to 15 days in seedling emergence. This delayed germination asks for an extra irrigation for seedling emergence, 7-10 days after sowing, which eventually increases the moisture level in the soil favouring growth of fungal pathogen like *Fusarium* which cause wilt incidence causing severe yield loss. To address this problem some preliminary work has been initiated at ICAR-NRCSS, Ajmer. Saxena *et al.*, (96) conducted an experiment to find out stimulatory effects of some known agro chemicals including potassium dihydrogen phosphate (0.5 and 1.0%), potassium chloride (2 and 4%), polyethylene glycol (10 and 20%) and thiourea (500 and 1000 ppm) on germination. The seeds were soaked for three time durations (12, 24 and 36 hrs) in dark at 25°C. Seeds primed with KH_2PO_4 (1%, 36 hrs), PEG (10 and 20%, 36 hrs) and distilled water (36 hrs) showed more than 75 % increase in germination over non primed seeds. The time taken in seedling emergence also reduced significantly as compared to non-primed seeds. The experiment was repeated with the same lot of seeds after 30 days of priming to see whether the effect of priming is stable or not, wherein no difference was observed. Seed priming is a seed treatment which improves seed lot performance under various environmental conditions like biotic and abiotic stresses. Many researchers have reported the beneficial effects of seed priming in cumin. Priming allows some of the metabolic processes necessary for germination to occur without germination take place (Rahimi, 87). Neamatollahi *et al*., (79) conducted experiments on hydro-priming and osmo-priming effects on cumin seeds germination. The treated seeds (control, hydro-priming and ZnSO4) of cumin were evaluated at germination and seedling growth for tolerance to salt (NaCl and Na_2SO_4) conditions at the same water potentials of 0.0, -0.3, -0.6, -0.9 and -1.2 MPa. Results revealed that inhibition of germination at the same water potential of NaCl and Na_2SO_4 resulted from salt toxicity rather than osmotic effect. Hydro-priming increased germination and seedling growth under salt stress.

In an experiment Sharma *et al*., (103) investigated the effects of application of some biological agents on cumin seeds and their impact on seed germination, promotion of seedling growth and wilt incidence. Bio control agents (BCAs) *Trichoderma viride, Aspergillus versicolar* enhanced seed germination and also seedling growth indices including root length, shoot length, weight of seedlings and reduced wilt incidence. In bio priming, the BCAs were applied to the seed improved by the efficacy of BCAs and the highest amount of shoot length (6.55 cm), and shoot weight (0.14 gm) was reached with the application of *T. viride*. Similarly, Sharma *et al*., (104) tested nine *Trichoderma* isolates as seed priming agent on cumin cultivar RZ 209. The result showed that *T. asperllum* (CuTa7-02, CuTa3-01), *T. koningiopsis* (CuTk7-01) and *T. harzianum* (CuTh9-02, CuTh3-03, CuTh8-01) significantly reduced wilt incidence (58-85%) and can be used as biological components in integrated

management of cumin *Fusarium* wilt.

Shivran *et al.*, (105) found positive effect of plant growth promoting bioformulations FK14 (*Pseudomonas pituda*) and FL18 (*Microbacterium taraoxidens*) as seed coats for obtaining higher growth and yield in cumin compared to control, however, better results were obtained with treatment of FK14 and FL18 in combination.

A combination of hydro matrix priming on two popular genotypes of cumin viz. GC4 and RZ209 showed very effective response under laboratory and field conditions (Saxena *et al.,* 97). The treatment of six hours hydro-priming followed by 72 hrs matrix priming with synthetic soil proved best in hastening germination in 90% seeds, germination occurred on 4^{th} day after inoculation as compare to 8^{th} day under control conditions. Genotype GC 4 was found to be more responsive to priming than RZ 209. Similar results were obtained under field conditions. The outcomes of study revealed that seed priming can not only hasten germination but can also be a cost effective technology and moreover can be performed by farmers. Priming also save one irrigation *i.e.*, second germination which is essential to enhance germination.

Shoor *et al.*, (106) investigated the effect of salinity priming on germination and growth stage of cumin. Results indicated that seed priming with saline media significantly increased germination percentage, seedling weight, germination rate, root length and plumule length. Salinity priming significantly increased dry matter accumulation and relative growth rate (RGR) and could be a useful strategy to increase the salt tolerance of cumin.

Seed priming has been used to improve the germination performance under temperature and water stress. Rahimi (87) evaluated the effects of osmo-priming (–0.8 and –1.2 Mpa) on vigour and germination performance in cumin at different temperature incubations under drought stress. The results showed that osmo-priming with PEG6000 solution accelerate seed germination to the largest extent and improved the germination rate and the uniformity of germination under drought stress especially in 15°C incubation compared to 10 and 25°C. This treatment also improved stress tolerant by improving germination performance at 10, 15 and 25°C and under water stress of –0.4 and –0.8 Mpa.

Genotype (G) and environment (E) effects on the seed biochemical constituents

Numerous studies have been conducted on the effect of production practices such as nutrition, harvesting time, plant densities (El-Sawi and Mohamed, 43; Kan *et al.*, 62; Azizi and Kahrizi, 13; Zolleh *et al.*, 121) and genotypes (Martos *et al.,* 73) on cumin seed yield, essential oil ratio and essential oil compositions. The essential oil yield and content of a plant may be influenced by nitrogen fertilizer. Randhawa *et al.*, (91) observed a decrease of oil yield of dill by increasing the nitrogen rate. Recently, Ashraf *et al.*, (10) studied the effect of nitrogen fertilization on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa*). They reported that the seed essential oil content did not vary with the change in applied N level but the major component of the oil, p-cymen, increased at 30 kg N/ha. Ahmadian *et al.*, (3) indicated that using manure increased seed and biological yield of cumin under drought conditions.

Research conducted on water and fertilizer requirements in cumin clearly shows that there are low requirements of fertilizers compared to other crop (Kafi, 59). Recently, Hashemi *et al.*, (50) observed an increase of the oil yield by N fertilization up to 60 kg ha^{-1} level and a decrease at higher N levels. Increasing nitrogen fertilizer caused a slight and insignificant decrease in the cuminaldehyde yield of the seed. Plant density also effects growth, development and seed yield of cumin plants. The effect of plant density was also studied by Hashemi *et al.*, (50). Apparently, plant density did not show any considerable effect on the oil yield. Similar outcome was reported by Azizi and Kahrizi (13) where plant density apparently did not show any considerable effect on the oil. In contrast, Erden *et al.*, (44) reported that different seed amount and inter row spacing affects the yield, yield components and essential oil composition of cumin. They also found that essential oil yield, essential oil ratio, 1000-seed weight, number of seeds per umbel, number of branches per plant, number of umbel per plant and essential oil components ratio were significantly affected by seed amount and inter row spacing. Felabi (46) reported that fall sowing dates of cumin could significantly increase dry matter accumulation, and improve seed yield by increasing growth period length compared to spring sowing dates in central parts of Iran. Recent study showed that sowing date and different ecotypes of cumin have the variable potential for survival percentage, yield components, biological and seed yields (Nezami *et al.,* 81). Though, sowing dates had no appreciable effects on thousands seed weight of cumin (Ehteramyan *et al.,* 42; Mershekari, 76) which could lead to the conclusion that this factor is under genomic control.

Genetic improvement programs-selection, mutation breeding, tissue culture and biotechnology

Selections

Available literature indicates that genetic base in the germplasm particularly for economic traits viz. yield, quality (volatile oil content) and reaction to different biotic and abiotic stresses is narrow in all the seed spices (Sharma, 102). This is also true for cumin, an allogamous species, as yield potential of the cumin is generally low. Crop also suffer from lack of usable variation for important yield traits and disease resistance in the germplasm collection and even if present may not be used with ease on account of very small size of their flower, thus restricting the crop improvement programs. The chromosomes of the different varieties have morphological similarities and there is no distinct variation in length and volume (Sastry and Anandaraj, 94). Moreover, the open canopy of cumin is another problem as a result only a low proportion of the incoming light is absorbed. The Leaf Area Index (LAI) of cumin is low (approximately 1.5). This might be a problem because weeds can compete with cumin for essential resources such as water and light and thereby lower yield. The slow growth and a short stature of cumin favours weed competition additionally. Genetic enhancement of cumin is possible only through accumulation of variability in the form of germplasm collected locally or procured globally as exotic germplasm (Parashar *et al.,* 85). So far ten varieties namely S 404 (1952), MC 43 (1970), GC 1 (1983), RZ 19 (1988), RZ 209 (1995), RZ 345 (2008), GC 4 (2006) were released by indigenous selection from available and collected germplasm of cumin while GC 3 (2000) was identified from exotic collection

Mutation Breeding

Another way of genetic enhancement and variability creation in cumin is mutation breeding. In the study conducted by Koli and Sharma (67) cumin seeds were subjected to gamma radiation levels of 20, 30, 40, 50 and 60 krad before planting. M_2 generation was produced by self-pollination of M_1, and 11 traits including yield were recorded. Some M_2 lines showed improvements in number of days to flowering, days to maturity and individual plant weight. Though, none of the progeny met the criterion of desirability in M_2 generation for yield/plant, may be because yield is a complex character and is a multiplicative product of several traits. Nevertheless, several desirable progenies were found for days to flowering, days to maturity and test weight.

Likewise, in another study carried out by Koli and Sharma (67), dry seeds of cumin (var. RZ19) were treated with 20, 30, 40, 50 and 60 krad gamma radiation. Mean plant height increased for all irradiation levels. However, its variance decreased (except in 30 krad treatment). Mean and variance of number of umbells and number of seeds per umbells increased linearly with irradiation levels but seed yield and 1,000-seed weight decreased. Based on the observations, 60 krad was tagged as the LD-50 dose, but lower doses *i.e.,* 40 krad and 50 krad also showed effective mutations for creating variability.

Recently, Ramkrishna (90) applied both gamma irradiation and chemical mutagens (EMS and sodium azide) for creation of variability in cumin, coriander, fennel and fenugreek. Mutagenic efficiency varied noticeably between crops and mutagens; gamma rays were relatively more potent on cumin as compared to chemical mutagens whereas on fennel it was just reverse. Efficient mutagens more often yielded superior M_2 progenies in cumin i.e. progenies having significantly higher yield than their parent. Seed yield per plant of M_2 progenies varied to different extents e.g. mutants showing yield advantage of 269 % were observed in cumin.

Sastry and Sharma (95) exposed dried seeds of RZ-19 with gamma rays (200, 300, 400, 500 and 600 Gy). No specific morphological mutations were observed in M_1 generation. A total of 50 normal looking plants from each radiation dose and control were advanced to M_2. Out of these 50 plants, 25 were self-pollinated to produce 'self-pollinated' M_2 progenies in each radiation dose. Seeds harvested from each of the 125 self-pollinated and open pollinated progenies were sown to rise M_2 generation. Three plants with white flowers were observed in contrast to pink flowers in RZ 19. Higher estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability in radiation treatments than in control indicated induction of heritable variation. Linear increase in mean values along with the radiation gradient was observed for most of the characters. 300 Gy and 400 Gy were found to be best doses which induced more mutations per dose; 200 Gy dose was ineffective while 600 Gy induced more of seed sterility.

Seeds of cumin varieties RZ 19 and RZ 209 were exposed to varying concentration (0, 0.25, 0.50 and 0.75%) of ethyl methane sulphonate (EMS) and colchicine solutions (Solanki *et al.,* 113). In M1/C1 generation, seed germination was reduced significantly in two concentrations i.e. 0.50 and 0.75% of EMS and in all the three concentrations of colchicine whereas 1000

seed weight increased significantly in two concentrations of colchicine i.e. 0.25 and 0.50% but no effect was observed in EMS treatments as compared to respective check. Variations for plant height (cm), branches plant^{-1}, umbellets umbel^{-1}, seeds umbellet^{-1}, 1000-seed weight (g) and seed yield plant^{-1} (g) were recorded in M2/C2 generations. The progenies derived from colchicine treatments had recorded higher means for seed yield per plant, plant height and branches per plant. A few progenies exhibited high coefficient of variation over the control along with high mean hence these may resulted in desirable segregants in their progenies facilitating selection.

The mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate, methyl methane sulphonate and sodium azide and their combinations were estimated by Yadav and Krishna (117) on two cumin genotypes, RZ 19 and RZ 209. In case of their sole treatments, the germination reduced linearly with increase in the doses. Gamma rays severely affected the germination. Based on reduction in germination the LD_{50} values for chemical mutagens appeared to be slightly less than 0.6 % for ethyl methane sulphonate, 0.06 % for methyl methane sulphonate and 0.6 mM for sodium azide. Chemical mutagens either as sole or as combined treatments had less severe effect on plant survival than gamma rays. Pollen sterility ranged between 10 to 22%. Gamma rays caused more sterility than chemical mutagens. The efficiency of mutagenic treatments showed that on RZ 209 gamma rays was most efficient at 70 KR whereas on RZ 19 it was 60 KR. So far two varieties namely GC-2 (1992) and RZ-223 (2004) have been developed through mutation breeding approach.

Tissue culture and genetic transformation

Production of cumin is limited by effect of several stress factors of which wilt (*Fusarium oxysporum* f.sp. *cumini*) and blight (*Alternaria bursnsii*) (Agarwal, 2) are the most serious. Conventional breeding methods to develop resistant varieties are handicapped by high constrains in performing recombination breeding approach and also due to low availability or absence of resistance source in cumin gene pool (Shukla *et al.,* 107). In such condition it is a pre-requisite for applications of biotechnological/ cellular genetic manipulations to develop resistance in cumin against biotic and abiotic stresses. Therefore, there is an increasing interest towards *in vitro* regeneration of cumin and further genetic manipulation of regenerated plants. Tissue culture can be used to create somaclonal variation especially under desired biotic and abiotic stress pressure for isolating resistant or tolerant plant types. On other hand tissue culture is also essential for genetic transformation for producing transgenic cumin plants with high stress resistance. The current available tissue culture protocol may be more compatible with variant cell induction and selection strategies of improvement. Furthermore, *ex vitro* survival of tissue culture derived plants are generally low in cumin and other herbaceous umbelliferous plants, which is a challenge in taking up tissue culture approaches.

The most reliable and efficient alternative to conventional breeding, is the *in vitro* propagation of cumin using different pathways (somatic embryogenesis and shoot regeneration). Regeneration has been achieved successfully in various members of Umbelliferae *viz*., *Daucus carota* (Kamada and Harada, 61), *Foeniculum vulgare* (Hunault, 55), *Eryngium* (Daniel *et al.,* 33) and among these *D. carota* has become a classical example of *in vitro* regeneration especially through somatic embryogenesis. Cumin, however appears to have limited potential for *in vitro* manipulation. Dave and Batra (35) were the first to study the somatic embryogenesis in cumin. They reported that among root, hypocotyl and cotyledon, hypocotyl on MS medium inoculated with 8 mg l^{-1} of BA was the source to somatic embryos. Later on, Hussein and Batra (56) also reported *in vitro* embryogenesis from hypocotyl segments. In another communication by Gupta and Bhargava (49) shoot organogenesis from hypocotyl explants in RZ 19 by the use of thidiazuron (TDZ) was found to be best. Regeneration was achieved with a frequency up to 30% on 0.5 and 0.1 mg 1^{-1} concentration of TDZ. Shoot regeneration in cumin through indirect somatic embryogenesis and indirect shoot organogenesis in callus culture derived from seedling explants such as hypocotyl and internodal stem segments have also been reported (Azza and Noga, 14). Azza (16) were able to regenerate cumin seedlings from callus formed from cotyledon and hypocotyl explants. However, other results showed better performance to hypocotyl compared to cotyledon explants (Shukla *et al.,* 107). Callus induction and establishment were studied using different explants of 12 diverse genotypes of cumin (Shukla *et al.,* 107). Among the different explants studied, hypocotyl gave maximum callus induction on semisolid MS medium supplemented with IAA and Kn except in genotypes, GC 1, MC 43, JC 19 and Hairy cumin wherein maximum response was obtained in cotyledon explant without any sub-culturing. On transfer to MS medium containing reduced levels of IAA and same Kn, the embryoids developed into heart shaped

and torpedo stages. Azza and Noga (15) obtained cumin somatic embryos in 7 days after transferring the cell suspension into liquid medium lacking plant growth regulators. In comparison to explants from hypocotyls and leaves, callus of hypocotyls had better growth.

A rapid and efficient method for regeneration of plantlets through embryo culture was reported by Ebrahimie *et al*., (39). This method yielded a large number of shoots within short period of time (30–50 days) without any subculturing. Direct shoot regeneration in tissue culture from mature embryo of diverse sets of cumin genotypes is a rapid and genotype-independent pathway (Ebrahimie *et al.,* 40). Similarly, Ebrahimie *et al*., (41) reported direct regeneration protocol in tissue culture of different genotypes based on pre-existing meristem. This study supports the feasibility of combining direct regeneration protocols using embryo and node of cumin for germplasm conservation by in vitro cloning and genetic improvement programme.

Adventitious shoot proliferation from aseptically germinated seedlings of cumin was reported by Mann *et al*., (72). Recently, Jakhar *et al*., (57) described *in vitro* regeneration in cumin and found that the shoot apex and hypocotyls explants responded differently to various concentration of cytokinin and auxin used alone or in combination to induce callus formations. Profuse callus induction was observed in shoot apex explant at 0.3 mg l^{-1} BAP and this callus remained potent for longer time. The hypocotyl derived callus also showed globular embryo like structure on 1 mg l 2^{-1}, 4-D. However, it did not regenerate into complete plant even after various manipulations. Shoot morphogenesis was observed in shoot apex derived callus cultured upon three subcultures on 0.3 mg l^{-1} BAP. Regenerated shoot induced roots within 3 weeks when cultured on 1 mgl^{-1} NAA.

Establishment of a stable embryogenic cell suspension (ECS) culture is a prerequisite for many biotechnological breeding methods. Opposed to conventional breeding, it is an alternative and attractive system for mutant selection and mass propagation at the cellular level, especially when the improvement of one or two easily identifiable characters is desired in an important variety (Lestari, 70). In cumin, Ahmed *et al*., (4) established ECS cultures from hypocotyl segments-derived embryogenic calli. After culture for 2-4 months on B5 solid callus induction medium (0.88 mg l^{-1} 2, 4-D + 0.86 mg 1^{-1} kinetin), friable ECS were induced. Plating of ECS (1-12 months old) on 3 different solid B5 media resulted in the induction and development of approximately six, two and six compact organized calli ml^{-1} of ECS on B5z, B5K and B5m media, respectively. Variation in callus induction ability was influenced by the time elapsed after subcultures and the medium used. Plated cells responded best after 5 days of subculturing; 11 calli ml^{-1} ECS were obtained while 8.2 shoots/ml ECS regenerated on B5zK medium containing 0.065 mg/l Zeatin + 0.021 mg l^{-1} Kin. A total of 230 plants were obtained, 75% of which survived under *ex-vitro* conditions, flowered and produced normal seeds. Chromosome number of suspension cells ranged from 12-28 chromosomes, and the majority of cells (51%) had a normal (14) chromosome number, which was also observed in 63% of tested root tip cells of regenerated plants.

Very little or negligible attention has been focused on the transformation potential of cumin so far. Singh *et al*., (111) from CSIR–Central Salt and Marine Chemicals Research Institute (CSMCRI), Bhavnagar, India, were first to established a convenient method of genetic transformation in cumin, with embryos as explant using biolistic gene gun method. In this study, pre-cultured cumin embryos were bombarded under 27 inches Hg vacuum, 25 mm distance from rupture disc to macrocarrier, 10 mm macrocarrier flight distance using 1100 psi rupture disc and 9 cm microprojectile travel distance. Shoot tips and roots of T_0 plantlets exhibited GUS expression after 3 months of bombardment. T_0 transgenics were confirmed with PCR amplification of 0.96 (*hptII* gene) and 1.3 kb (*gus* gene) fragment, while southern blot was done using PCR amplified DIG labeled *hptII* gene as probe. As compare to biolistic method, *Agrobacterium* transformation methodology offers several advantages, including a low copy number of gene insertion into the plant genome, high stability, precise transgene integration and high efficiency. Later on, researchers from CSMCRI, established an efficient and reproducible method of *Agrobacterium*-mediated genetic transformation in cumin for the first time with a transformation efficiency of 1.5% (Pandey *et al.,* 83). In this study, around 1020 embryos were used for the optimisation of transformation conditions. These conditions were bacterium suspension of 0.6 OD_{600}, co-cultivation time 72 hrs, acetosyringone 300-μM and blade based wounding of explants. T_0 plantlets showed β-glucuronidase expression and gene integration was confirmed as per the method given by Singh *et al*., (111).

Variability studies – Phenotypic and molecular

Crop improvement methods are based on the selection of desirable genotypes with genetic variation, therefore knowledge of genetic diversity is a main prerequisite and the first step in plant breeding (Allard, 6). Traditionally, diversity is determined by evaluating differences in

phenotype and morphology. However, morphological determinations need to be taken by an expert in the species as they are subject to changes due to environmental factors and may vary at different developmental stages. Moreover, the morphological characters are limited in number (Kalia *et al.,* 60). Unfortunately, the potential genetic diversity available to conduct conventional breeding for resistance against biotic and abiotic stress factors is limited in cumin (Champawat and Pathak, 29). However, with limited variability, studies on genetic relationships among cumin ecotypes by means of morphological traits (Avatar *et al.,* 11; Singh *et al.,* 110; Bahraminejad *et al.,* 22), variation in salinity response (Singh *et al.,*110; Dhayal *et al.,* 37) as well as yield and growth traits (Baswana *et al.,* 24) have been reported previously. Study of Avatar *et al*., (11) to estimate genetic divergence through D^2 technique among 30 genotypes with 13 morphological traits grouped genotypes into five clusters, of which clusters I and II were closest. As per their assumption, they pointed out, that the variation within the germplasm is limited. In contrast to this, Baswana *et al*., (24) reported significant and wide genetic variation in 50 diverse cumin genotypes and found high phenotypic coefficient of variation for yield per plant and seed per umble. Similarly high phenotypic coefficient of variation was also observed by Mathur *et al*., (74) for yield per plant.

Results of Singh and Jadeja (109) on 25 genotypes showed sufficient variability among the agromorphological traits *viz*., grain yield per plant, flowering duration, plant height, branches per plant, umbels per plant, seeds per umbel, biological yield per plant, harvest index and test weight; while days to 50% flowering, days to maturity and umbellets per umbel showed narrow range of variability or absence of variability in genotypes for these traits. These findings of narrow range of variability or absence of variability are in agreement with Mathur *et al*., (74) where days to flower and days to maturity showed a low variation. Regarding biochemical parameters Singh and Jadeja (109) found sufficient variability for essential oil content. Hashemian *et al*., (51) also indicated significant differences between different accessions from northeast of Iran for essential oil yield. Recently, in a germplasm set of 160 genotypes, Bairwa *et al*., (23) observed high GCV for seed yield per plant followed by plant height, primary branches per plant and test weight. These results are in congruence with Solanki and Joshi (112) and Dhayal *et al*., (37) for high GCV for plant height, test weight and seed yield per plant in cumin.

Study of morphological traits is time and energy consuming and costly, and usually coupled with environmental effects for gene expression. Further the lack of information on genetic diversity and intraspecific relatedness in cumin has offered limited scope for genetic improvement, in addition efforts for effective conservation and management of germplasm resources were not much emphasised (Parashar and Malik, 84). During the past decades, classical methods to evaluate genetic variation have been complemented by molecular techniques. Applying DNA molecular markers, for assessing genetic diversity in plants with low genetic diversity have shown advantages over phenotype based variability assessment system. Markers are neutral, independent to age, sex and tissue type, not affected by the environmental conditions, cost effective and are more informative than morphological markers (Da Mata *et al.,* 32). A lot of work has been done on cumin's classical breeding, industrial and pharmaceutical applications, but marker based genetic diversity is less characterized. Despite their utility for elucidating genetic relationships within plant species very limited molecular studies have been done on characterization and fingerprinting of cumin at both national and international level. Though, during past years, few efforts have been made to analyse the genetic diversity of cumin and many marker systems especially dominant marker like RAPD, ISSR, AFLP have been employed.

Bahraminejad *et al*., (21) used 23 RAPD primers to find the genetic variation among 49 cumin ecotypes which are sub-populations belong to nine populations from different Iranian provinces. Of the 23 random primers screened, 18 primers yielded 252 amplicons. Polymorphism information content (PIC) values for studied RAPD markers with Power Marker version 3.25 varied from 0.075 to 0.37 with an average of 0.31. Cluster analysis using UPGMA in NTSYS-pc among the populations grouped the genotypes into two groups at the similarity level of 0.43 with three sub-classes in cluster two. Hence the study showed arrangement of markers randomly on the genome and existence of potential variability in Iranian cumin.

Hossein *et al*., (54) used 13 RAPD primers and 22 ISSR primers to study genetic relationships among 42 genotypes of cumin of Iran. Overall, the banding patterns of 22 ISSR primers and 13 RAPD primers revealed 202 (67.32%) and 85 (54.90%) polymorphic bands, respectively. The range of similarity coefficient in ISSR and RAPD markers were 0.48-0.92 and 0.25-0.94, respectively. Centroid based cluster analysis in NTSYS-

pc generated 8, 7, 6 and 3 groups from ISSR, RAPD, ISSR+RAPD and morpho-agronomic data, respectively. During pooled analysis of marker data (ISSR+RAPD), Jaccard similarity matrix showed a range from 0.45 to 0.90. Correlation value of Mantel test for comparisons of similarity matrices was 0.541.

Study of RAPD based diversity analysis on 32 genotypes of cumin by Baghizadeh *et al.*, (18) produced 154 bands, of them about 86% were polymorphic. Cluster analysis based on UPGMA method and Dice's similarity coefficient in NTSYS-pc software gropued all genotypes into six cluster at 46% similarity coefficient.Based on RAPD analysis of 12 varieties with 400 primers, Parashar and Malik (84) observed 321 (63%) polymorphic loci from 55 polymorphic primers; hence resulting in a polymorphism frequency of 63.18% and an average of 5.8 polymorphic bands per primer. Similarity matrix values using Jaccard coefficient based on RAPD markers ranged from 0.37 (UC 299/RZ 223) to 0.87 (RZ 19/RZ 209). Dendrogram showed two major clusters, smaller one having one variety (RZ 223) and the larger cluster that could be further divided into 8 different sub clusters. Likewise, out of 100 ISSR primers, 27 primers, out of 39 amplified, gave polymorphic bands. These polymorphic primers produced 305 bands of which 169 (55.40%) were polymorphic with an average of 6.2 polymorphic bands per primer. The similarity matrix values using Jaccard's coefficient based on ISSR markers ranged from 0.63 (UC 239/RZ 223 and UC 274/UC 331) and 0.94 (RZ 19/RZ 209). On same 12 genotypes, Parashar and Malik (84) also carried out study with 36 start codon targeted polymorphism (SCoT) marker system (Collard *et al.,* 31). From 32 amplified Scot primers, 577 (79.80) were polymorphic. The similarity matrix values using Jaccard's coefficient based on SCoT markers ranged from 0.24 (UC 225/CUM 3 and UC 274/CUM 3) and 0.79 (RZ 19/RZ 209).

Ma *et al.*, (71) detected genetic diversity in 24 genotypes of cumin using 12 ISSR markers. Total 118 alleles were revealed with the average of 9.42 alleles per ISSR marker, the number of alleles per primer pair ranged from 6 to 16. The value of PIC ranged from 0.11 to 0.62 with a mean of 0.35. The genetic similarity index among 24 varieties varied from 0.33 to 0.90, with an average of 0.73 which indicated high diversity among 24 varieties. Cluster analysis grouped all cultivars into six clusters irrespective of their geographical area of collection.

Genetic diversity has also been investigated by AFLP markers. AFLP markers were used to evaluate the genetic variation within and among 15 lines of green cumin (*C. cyminum*) and 3 populations of white cumin *C. setifolium*). From 6 primer combinations (EcoRI/MseI), 149 bands were scored of which 73 (49%) were polymorphic. The largest numbers of polymorphic bands (20 bands) were produced using primer combination EcoRI-AGT/MseI-CCG, and the lowest numbers of polymorphic bands (3 bands) were produced by primer combination EcoRI-ACT/MseI-CGG. Genetic diversity of *C. cyminum* (0.150) was more than *C. setifolium* (0.084), while the variation between species was more (0.163) than the diversity within species. UPGMA based dendrogram, completely distinguished these species in genetic distance of 45%. This study revealed that genus *Cuminum* poses relatively low level of variation due to its self-pollinating nature, and white cumin as a wild type may be used as a new source of genetic variation for performing pre-breeding approaches and hybrid production (Kermani *et al.,* 64).

In another study with AFLP of 20 lines of Iranian cumin, Kermani *et al.*,(65) found 222 scorable bands 8 primer combinations (EcoRI/Mse.1) with 23% (51 bands) polymorphic bands. The maximum number i.e. 13 polymorphic bands were produced from primer combination EcoRI-ACG/MseI-CGG while it was least (2 bands) with EcoRI-ACT/MseI-CGG. The PIC for each primer combination was ranged from 0.20 to 0.45. Also the pair-wise genetic distance was from 0.081 to 0.98. The dendrogram constructed using UPGMA method, distinguished 4 main groups among 20 lines of cumin which was also confirmed by multidimensional analysis.

Bahraminejad *et al.*, (20) assessed genetic variability in 49 cumin ecotypes of Iran using AFLP markers. Six primer combinations, (EcoRI/MseI) produced 126 bands of which 60 were polymorphic. The primer combinations E+AGC/M+CTC and E+AGT/M+ CGG showed the highest and lowest polymorphic bands, respectively. PIC values based on obtained molecular data varied among all ecotypes, highest (0.55) and the lowest (0.27) PIC value belonged to E+AGT/M+CGG and E+AGC/M+CTC, respectively. Result showed Yazd and Semnan populations had the highest difference with 8 polymorphic bands whereas Esfahan, Khorasan-Razavi and Kerman were the closest population based on differences in AFLP bands. Meanwhile E+AGT/M+CGG had the highest polymorphism in Pars and Yazd while E+AGC/M+CTC in Kerman and Golestan showed the highest polymorphism. Totally E+AGT/M+CGG had the highest polymorphism and can be recommended for recognition of ecotypes from each other. Molecular diversity among cumin populations showed Kerman, Esfahan and Khorasan-Razavi have the same origin.

Also it is expected that crossing of Semnan and Pars populations, which are further away can produce a powerful hybrid. Based on the obtained result, it can be concluded that there is a high potential of variability in Iranian cumin populations, which is a very important source for cumin breeding objectives.

Among various molecular marker system, microsatellite markers (SSRs) are becoming the markers of choice for marker assisted breeding, due to their high robustness and polymorphism. Due to their co-dominant and usually single-locus nature, SSR loci can be identified, and their alleles can be recognized in different varieties and genotypes of the same species and often in other closely related species also. But still no molecular tools have been developed for cumin breeding due to high marker development cost and labor-intensive process. Therefore, it is highly valuable to investigate the transferability of SSR markers from related species/genera to cumin (Kumar *et al.,* 68). Microsatellites in a perennial Apiaceae such as *Eryngium alpinum*, have the potential to provide a new insight on the genetic processes within and between populations (Gaudeul *et al.,* 47). Similarly, publicly available SSRs reported in carrot (Niemann *et al.,* 82; Cavagnaro *et al*., 28) and celery (Acquadro *et al.,* 1) will also give the advantages to identify transferable markers in cumin.

A set of 13 SSRs, developed in *Eryngium* genus of Apiaceae family (Gaudeul *et al.,* 47), were used to investigate the genetic variation between 49 cumin ecotypes of different provinces of Iran (Bahraminejad and Mohammadinejad, 19). PIC values varied between 0.18 - 0.37 and mean gene diversity based on Nei and Shannon was 0.37 and 0.54, respectively. Cluster analysis through NTSYS-pc2 grouped 49 genotypes in three classes at 0.71 level of similarity and evident that most populations were placed in the second class, which consisted of five subclasses. Average gene diversity based on Nei and Shannon was 0.37 and 0.54, respectively.

Kumar *et al*., (68) studied SSR marker transferability from carrot to cumin and reported successful amplification, 38% (19/50) of the tested carrot SSRs. Of these 19, 12 primers (63%) showed a clear and strong PCR product. The result was in agreement with Cavagnaro *et al*., (47) where transferability of SSRs across Apiaceae taxa was reported to widely vary among the accessions. The outcome of study of Kumar *et al*., (68) indicates that there is a potential for transferring SSR markers of carrot in cumin. Overall, it can be briefed that there is good scope for applying identified markers in assessing population genetic diversity, molecular breeding, evolutionary studies in cumin and other *Apiaceae* species.

Concluding remarks and futuristic directions

Cumin (*Cuminum cyminum* L.) is one of the most important pharmaceutical and economical crop species among umbellifereae plants. It is extremely valuable for its medicinal and nutritional properties and is a source of income, as a cash crop, for farmers in the dry regions of India, Iran, Syria, Pakistan, Turkey. Owing to its increasing demand in various food industries, it has become second most popular spice in the world after black pepper (Hashemian *et al.,* 51). But, it is a challenging task for the farmers to protect cumin crop from various biotic and abiotic stresses. Fig1 summarizes the uses, problems, current research status and innovative research approaches to be followed in cumin research programme. Although cumin is rained crop but susceptibility to frost especially at flowering and early seed formation stages; and fungal diseases *Alternaria* blight and *Fusarium* wilt is a major problem and detrimental factors in hindering successful cumin cultivation. Cumin seedlings are also sensitive to salinity. Heavy losses have been observed due to combined effect of chilling and frost injury in arid and semi-arid track of Rajasthan and Gujarat. Incidence of frost reduces production drastically hence causes serious loss in yield (Datta, 34). Due to vulnerability of cumin to frost and diseases like *Alternaria* blight damage causing 100% crop loss, its cultivation is always at high risk. But due to high price of cumin, farmers are willing to cultivate the crop considering the inherent risk of crop failure. So far no efforts have been made to identify the source of resistance against low temperature injury in available germplasm of seed spices crops. Since the resistance to disease & frost and improvement in quality has special significance in cumin, well planned efforts must be taken up to enhance the variability.

Availability of a broad genetic base and identification of superior germplasm with respect to phytochemical constituents are prerequisite for initiating crop improvement programmes for any plant (Kalia *et al.,* 60). Very scanty information has been generated in cumin based on morphological and biochemical markers. Similarly, few DNA marker based diversity studies have been carried out in this commercial crop. Only one report from Iran has documented the use of SSR markers for diversity analysis but there is further need to develop SSR marker to analyse the genetic diversity and further their use in cumin breeding. Co-dominant molecular markers like SSR and Single-nucleotide polymorphism

(SNP) will not only support diversity analysis but also be helpful in detection of major genes or Quantitative Trait Loci's (QTL's) for traits of interest through genomic assisted breeding and genome wide association studies (GWAS). Though, genetic transformation protocol using gene gun and *Agrobacterium* are developed for cumin, but still information lacks for transgenic genotypes with altered agronomic or other commercial traits like frost and disease tolerance. In present genomic era, next generation sequencing and various SNP genotyping platforms have paved the path for implementing molecular breeding and genetic engineering in many plants including horticultural crops for crop improvement. However, in comparison to other crops, genetic improvement of seed spices of family *umbellifereae* is limited and mainly restricted to carrot. Bearing in mind the high economic value of cumin, there is urgent need for multi-disciplinary (breeding-genomics-biochemistry) collaborative approach to develop high yielding resistant varieties with better quality with complete genetic and genomic analysis.

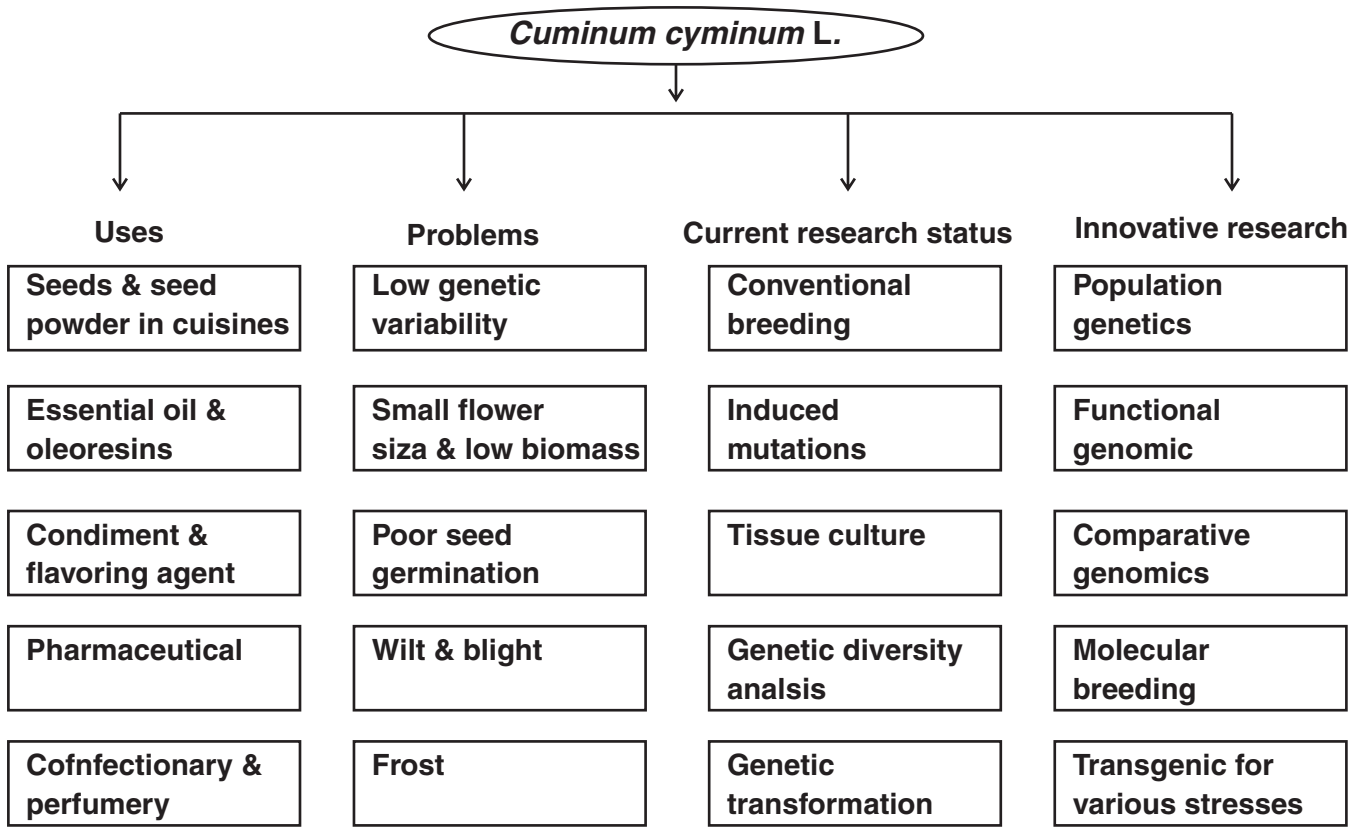

Fig 1: Uses, problems, current research status and suggested innovative approaches for cumin research programme

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