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GLYCOALKALOIDS IN PEELS OF INDIAN POTATOES

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Potato is the fourth most important crop worldwide after maize, wheat and rice and is rich source of proteins, carbohydrates, vitamins, minerals and phytonutrients. However, potatoes are also known to contain nitrogenous steroidal glycosides, called glycoalkaloids. Due to the toxicity and bitter taste conferred by glycoalkaloids, they are unwanted potato constituents for consumers. In potato tubers α-solanine and α-chaconine account for 95% of glycoalkaloids hence are generally referred as total glycoalkaloids (Soresen *et al.,* 2008). These alkaloids consists of a non polar lipophilic steroid nucleus that is extended by two fused nitrogen-containing heterocyclic rings at one end and bound to a polar water-soluble trisaccharide at the other end. Both solanine as well as chaconine share the same aglycone, namely solanidine, but differ in their carbohydrate component. Small amounts of aglycone and partial glycosides are normally present in potato (Smith *et al.,* 1996). Naturally occurring in potato tubers, peels, sprouts and blossoms, potato glycoalkaloids when found in tubers at high concentrations are reoported to cause poisoning and in a few instances death. Basically, glycoalkaloids are stress metabolites, arising in tubers, in response to excessive light, wounding, premature harvesting and other adverse conditions (Sinden and Webb, 1974). They are thought to function in the chemical defence of the plant, as non-specific protectors or repellents against potential pests and predators (Roddick, 1989). Glycoalkaloids are present throughout the potato plant with the highest levels observed in those parts of plant with high metabolic rates (Van Gelder, 1990). Tubers have much lower glycoalkaloids content with higher levels found in the periderm and cortex, decreasing markedly towards the pith but the distribution is not uniform (Dale *et al.,* 1998). Glycoalkaloids content of the foliage is also an important factor in protecting plants from fungi, insects and predators (Uppal, 1987).

In humans, glycoalkaloid poisoning elicits a wide variety of symptoms ranging from gastrointestinal disorders, through confusions, hallucination and partial paralysis to convulsions, coma and death. Chaconine and solanine are equally potent inhibitors of acetylcholinesterase responsible for hydrolysing the neurotransmitter acetylcholine, a key process in nerve impulse conduction across cholinergic synapse thus resulting in neurological symptoms. Ability of glycoalkaloids to disrupt sterol containing membranes is responsible for damaging cells in the gastrointestinal tract and also in other

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tissues or organs into which glycoalkaloids are transported following absorption. Keeping these in view, new potato varieties are checked for glycoalkaloids levels since there is a guideline stating that new potato varieties should not contain more than 20 mg of glycoalkaloids per 100 g of fresh weight. However, some researchers feel that the safe upper limit is much lower i.e 6 mg/100mg (Morris and Lee, 1984).

Indian potato cultivars have not been analyzed for the presence of glycoalkaloids in them, therefore, it was pertinent to determine glycoalkaloid concentration in the Indian potatoes being grown and consumed in the country. Since the content of glycoalkaloids are known to be high in peels than the peeled tuber, therefore, in the present study the peels have been analyzed for glycoalkaloids. A number of analytical methods have been reported for the determination of individual and total glycoalkaloids in potato tubers. These methods commonly employ extraction by aqueous base. The determinative steps involve thin-layer chromatography (Cadle *et al.,* 1978), gas-liquid chromatography (Herb *et al.,* 1975), high performance liquid chromatography (Morris and Lee, 1981), colorimetery (Wang *et al.,* 1972) or titrimetry (Fitzpatrick and Orman, 1974). In the present study a method was validated for the analysis of glycoalkaloids using HPLC and this method was used for the estimation of α-chaconine and α-solanine from peels of forty one Indian potato cultivars which are being grown in the different parts of the country.

The potato cultivars were raised at ICAR-CPRIC, Modipuram, India during *Rabi* season under standard package of practices. Freshly harvested tubers from mature crop of forty one potato cultivars were used. Tubers were washed and peeled (outer 2 mm layer) using manual peeler. Peels were finely diced, mixed and sampled for the analysis of glycoalkaloids *viz*. α-chaconine and α-solanine. Reagents for analysis were prepared from chemicals of high purity (HPLC grade) ultra pure water. The glycoalkaloid standards *viz*. α-chaconine and α-solanine were procured from Sigma (USA). Samples of peel (10 g each) were macerated in presence of 20 ml solution made of water: acetic acid: sodium bisulphate (+kieselguhr) in 95:5:0.5 ratio and were diluted up to 50 ml with the same extraction solution. Samples were cetrifuged at 5000 g for 10 min and supernatant was retained. Extraction cartridge C18 (Sigma-Aldrich, USA) was conditioned with 5 mL acetonitrile. Conditioning step was repeated with 5mL water: acetic acid: sodium bisulphate solvent. Conditioning step was repeated for one more time. 10 ml of supernatant (~2 g equivalent potato sample) was passed through the cartridge and the liquid was discarded. Cartridge was washed with 4 mL of water: acetonitrile (85:15 ratio). Glycoalkaloids were eluted with 4 mL of mobile phase i.e acetonitrile: phosphate buffer (0.022 M phosphate buffer, pH 7.6) and the final volume was raised to 5 ml with the mobile phase.

Mobile phase 'A' was acetronitrile and mobile phase B was 0.022 M phosphate buffer pH 7.6. The method of Hellenas, 1986 with modifications was standardized for the analysis of α-solanine and α-chaconine (Singh *et al.,* 2011). A reverse phase column (125 x 4 mm RP-18 Column, Merck, Germany) was used on the LaChrom HPLC system (Merck-Hitachi Darmstadt, Germany). A binary pump system was standardized for estimation of glycoalkaloids using Mobile phase A (Acetronitrile) and B (phosphate buffer) in the ratio of 55: 45, respectively. Flow rate of the mobile phase was 1.5 ml/ minute and injection volume was 20 µl. The retention time for α-solanine was about 3 min, and α-chaconine was about 4 min

(**Fig. 1**). The concentration of α-solanine and α-chaconine in the samples was identified and quantified using ESTD method and the standard curve was prepared using α-solanine and α-chaconine mixture in 1:1 ratio. All the analysis was performed in three replications and the data was subjected to statistical analysis using complete randomization block design (CRBD). The ANOVA was performed at 5% level of significance. The dendrogram of glycoalkaloids were analysed using XLSTAT software (XLSTAT version 2014.4.05).

In Indian potatoes, α-solanine content ranged from 0.14 (Kufri Kundan) to 1.86 mg/100 g FW (Kufri Chamatkar) with an average content of 0.76 mg/100 g FW (**Fig. 2**). α-solanine content differed significantly in Indian potatoes. Along with Kufri Kundan, peels of Kufri Chipsona-3 (0.24 mg/100 g FW), Kufri Chipsona-1 (0.31 mg/ 100 g FW), Kufri Sheetman (0.33 mg/100 g FW) and Kufri Kumar (0.34 mg/ 100 g FW) also exhibited low content of α-solanine. Cultivar Kufri Chamatkar contained the maximum content of α-solanine followed by Kufri Badshah (1.68 mg/100 g FW), Kufri Himalini (1.44 mg/100 g FW), Kufri Sherpa (1.27 mg/100 g FW) and Kufri Safed (1.21mg/ 100 g FW). In another study, α-solanine content has been found to range from 0.5 to 50.2 mg/100 g of raw potato peel of twelve varieties (Bushway *et al.,* 1983). The range of α-solanine content in the present study was much lower than this.

α-chaconine content ranged from 0.11 to 2.00 mg/100 g FW with the lowest values exhibited by Kufri Kundan and the highest by Kufri Neela (**Fig. 3**). The average α-chaconine content in peels was 0.86 mg/100 g FW. There was significant difference in α-chaconine content in Indian potatoes. Cultivars Kufri Muthu, Kufri Chipsona-1, Kufri Chipsona-3 and Kufri Jyoti also contained low content of α-chaconine as 0.28, 0.28, 0.34 and 0.38 mg/100 g FW, respectively. Whereas α-chaconine content was high in cultivars Kufri Badshah (1.95 mg/100 g FW), Kufri Chipsona-4 (1.88 mg/ 100 g FW) and Kufri Gaurav (1.81 mg/100 g FW). Bushway *et al.* (1983) reported a range of α-chaconine content from 1.3 to 56.7 mg/100 g in raw potato peel.

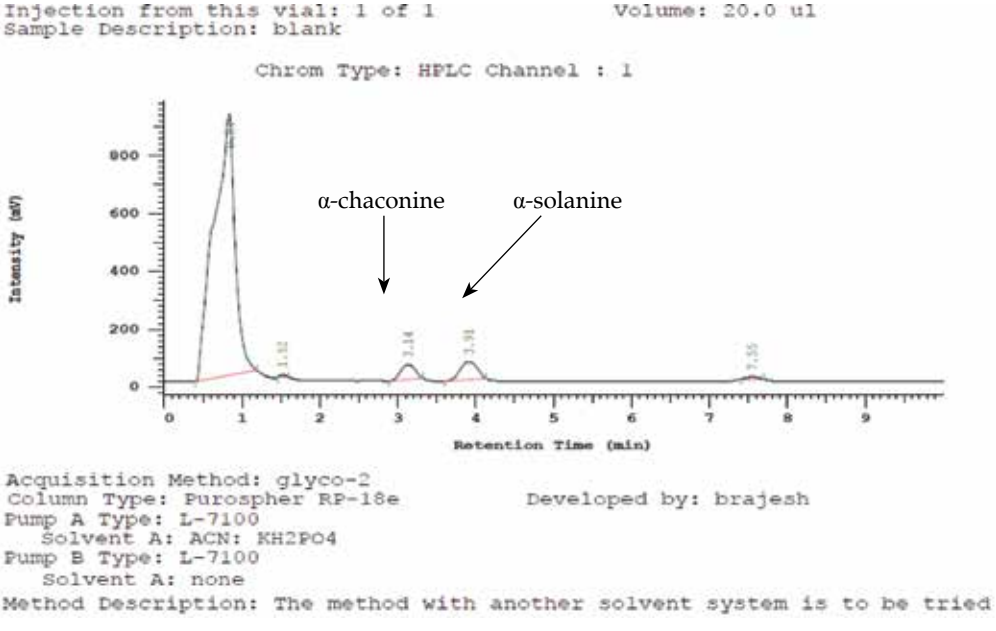

Fig. 1. HPLC Chromatogram of α-solanine and α-chaconine from peels of Kufri Megha.

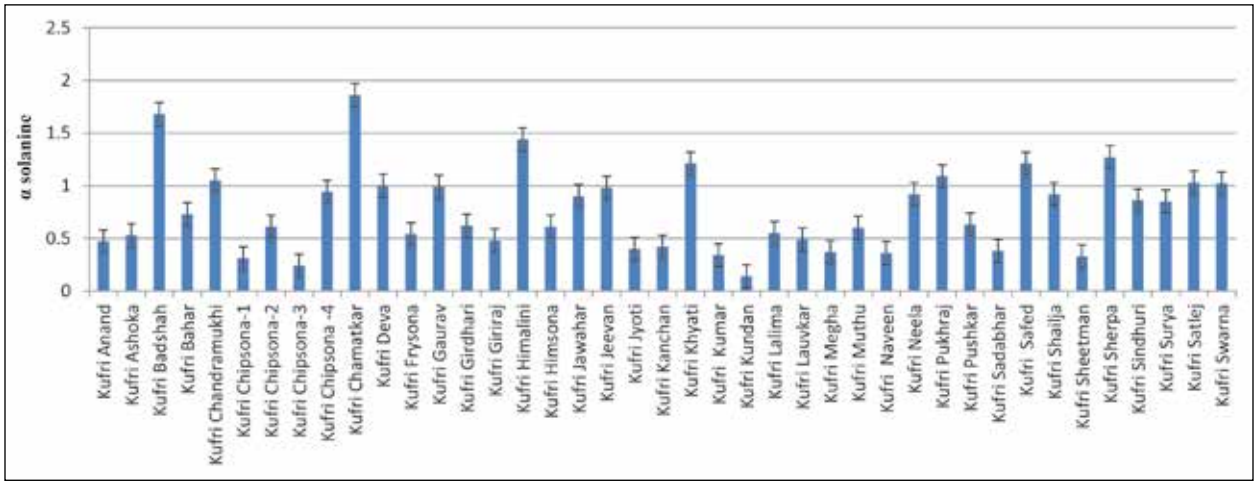

Fig. 2. α-solanine content (mg/ 100 g fresh wt.) in peels of Indian potatoes (Error bars represent SE values at 5% level of significance).

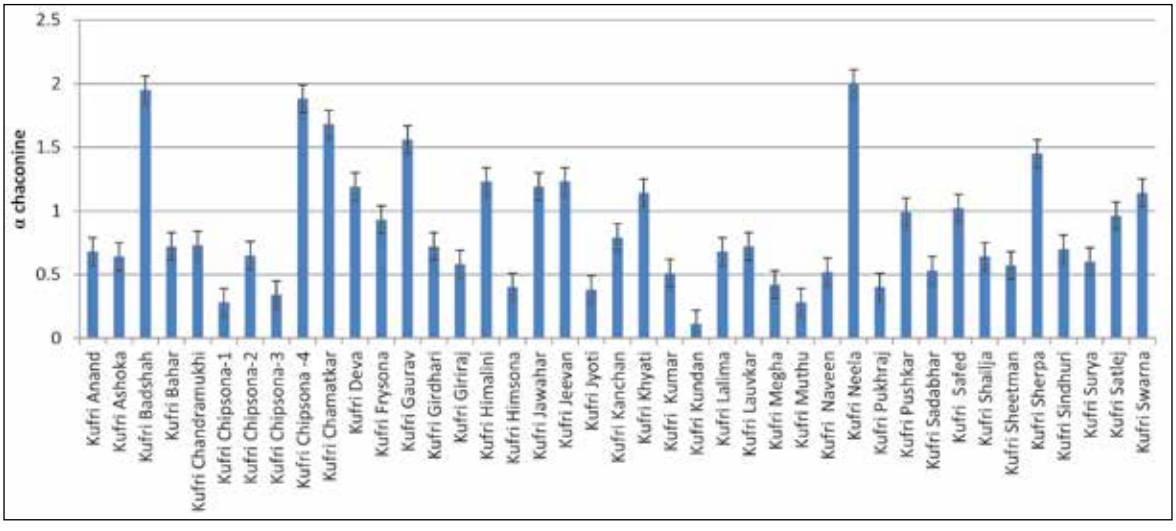

Fig. 3. α-chaconine content (mg/ 100 g fresh wt.) in peels of Indian potatoes (Error bars represent SE values at 5% level of significance).

Significant differences have been observed in total glycoalkaloids of Indian potatoes. The mean total glycoalkaloids content was 1.62 mg/100 g FW with the lowest values exhibited by Kufri Kundan (0.24 mg/100 g FW) and the highest by Kufri Badshah (3.62 mg/100 g FW). Cultivars Kufri Chipsona-1, Kufri Chipsona-3 and Kufri Jyoti also contained low contents of glycoalkaloids as 0.59, 0.58 and 0.78 mg/100 g FW (**Fig. 4**). Bushway *et al.* (1983) reported a range from 1.8 to 106.8 mg/100 g of total glycoalkaloids in raw potato peels. Total glycoalkaloids ranged from 197 to 518 mg/kg dry matter (equivalent to 19.7 to 51.8 mg/kg on dry weight basis) from stored tubers of three potato cultivars (Haase 2010). Bushway *et al.* (1980) determined α-chaconine and α-solanine content from flesh of five potato varieties stored at 3.3°C and 7.2°C for nine months. The content ranged from 3.89 to 23.04 mg/100 g and 1.69 to 12.51 mg/100

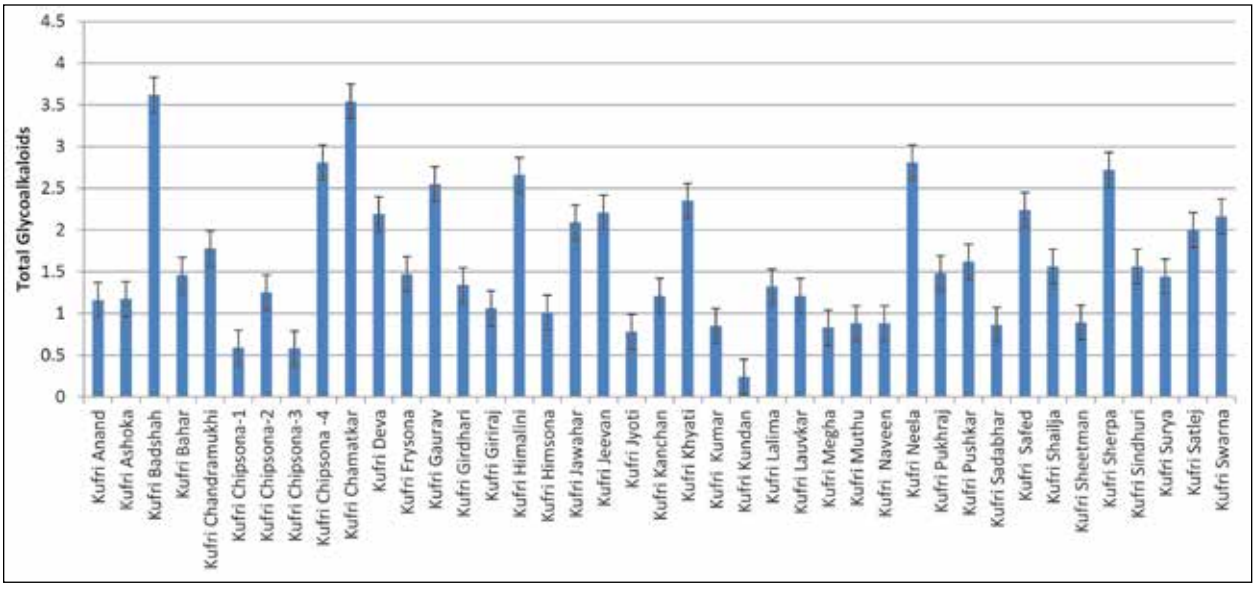

Fig. 4. Total glycoalkaloid content (mg/ 100g fresh wt.) in peels of Indian potatoes (Error bars represent SE values at 5% level of significance).

g, respectively. Peels are known to contain high contents of glycoalkaloids and the range of alkaloids was found to be much lower in peel of Indian potatoes. The possible reason for low glycoalkaloids in Indian potato peels might be that the tubers were harvested at full maturity.

Cluster analysis was carried out to find out group of cultivars containing high, medium and low content of glycoalkaloids. Cluster analysis of α-solanine and α-chaconine and total glycoalkaloids grouped the cultivars into 3 clusters (**Fig. 5**). Mean performance of cultivars in each cluster is depicted in **Table 1**. Lowest content of α-solanine, α-chaconine and total glycoalkaloids was exhibited by 26 cultivars *viz*. Kufri Sindhuri, Kufri Shailja, Kufri Surya, Kufri Bahar, Kufri Pukhraj, Kufri Pushkar, Kufri Frysona, Kufri Lauvkar, Kufri Anand, Kufri Ashoka, Kufri Kanchan, Kufri Giriraj, Kufri Girdhari, Kufri Chipsona 2, Kufri Lalima, Kufri Sadabahar, Kufri Naveen, Kufri Kumar, Kufri Sheetman, Kufri Megha, Kufri Jyoti, Kufri Muthu, Kufri Himsona, Kufri Chipsona 3, Kufri Chipsona 1 and Kufri Kundan which constituted cluster 1. Highest α-solanine, α-chaconine and total glycoalkaloids containing cultivars were grouped in cluster 2 that is constituted by seven cultivars *viz*. Kufri Neela, Kufri Chipsona 4, Kufri Gaurav, Kufri Sherpa, Kufri Himalini, Kufri Chamatkar and Kufri Badshah. Eight cultivars came under cluster 3 that contained moderate levels of α-solanine, α-chaconine and total glycoalkaloids. The analysis of glycoalkaloids in peels showed

Table 1. Means of glycoalkaloids in 3 groups formed by cluster analysis of 41 Indian potato cultivars.

Class	No. of cultivars	α-solanine (mg/100g FW)	α-chaconine (mg/100g FW)	Total glycoalkaloids (mg/100g FW)
Cluster 1	26	0.53	0.56	1.10
Cluster 2	7	1.30	1.71	2.96
Cluster 3	8	1.05	1.08	2.13

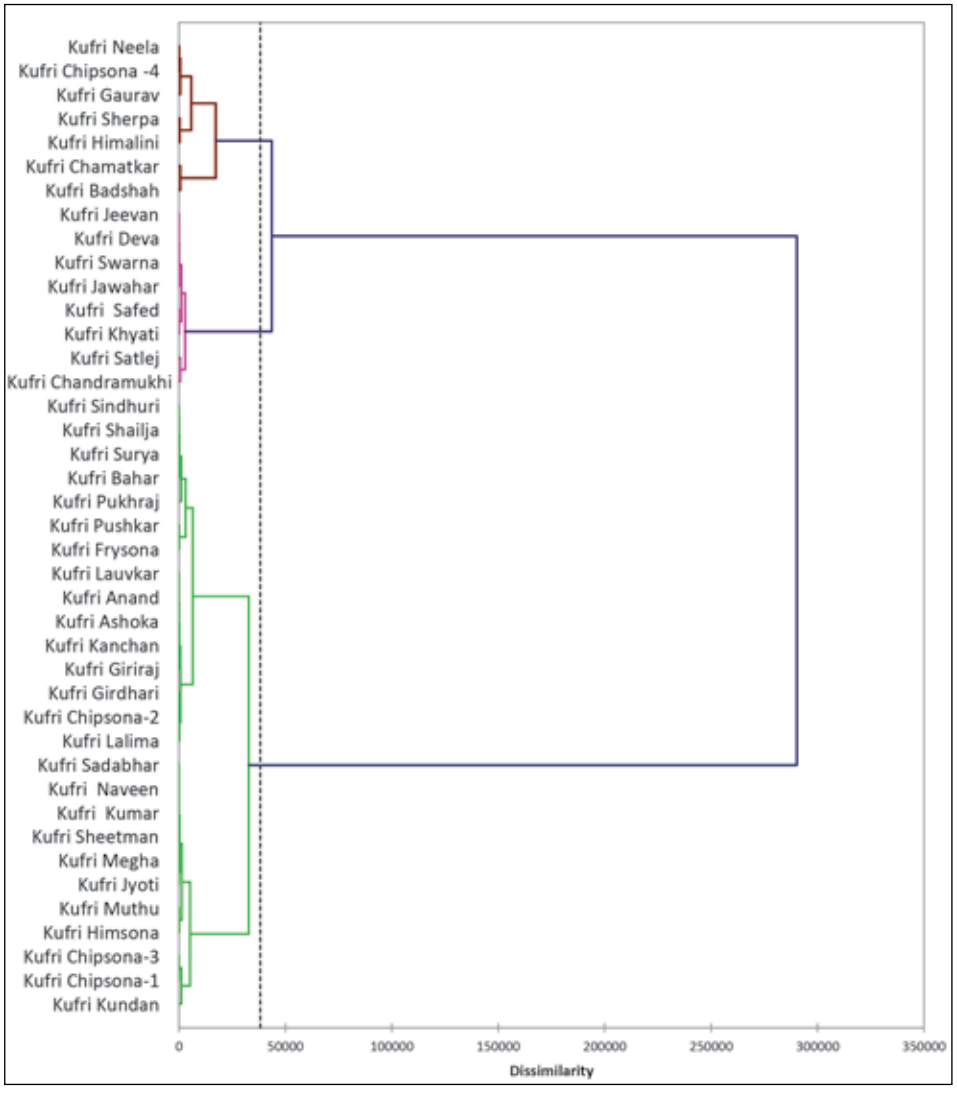

Fig. 5. Clustering of cultivars based on glycoalkaloids.

that Indian potatoes contain low concentration of glycoalkaloids. Besides, mostly potatoes are peeled and then used for consumption purposes in most of the Indian recipes, it is assumed that the health hazards from potatoes due to alkaloids is highly reduced. The persistence of these alkaloids in the processed and cooked potatoes needs further investigations.

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